UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OFPREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

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SUBJECT: **Atrazine**: Toxicology Chapter of the Reregistration Eligibility Decision. **REVISED**
Chemical number 080803.
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Attached is the revised **Atrazine** Toxicology Chapter of the Reregistration Eligibility Decision, based on Phase I Error Correction review by the Registrant.

Atrazine

PC Code: 080803

Toxicology Disciplinary Chapter for the Reregistration Eligibility Decision Document

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1.0 HAZARD CHARACTERIZATION

The toxicity data base for atrazine is adequate for selection of endpoints for use in risk assessment. The Hazard Identification Assessment Review Committee (HIARC) met on 5/4/00 and evaluated the acceptable studies available in the data base and established an acute and a chronic reference dose (RfD) as well as doses and endpoints for short-, intermediate- and long-term dermal and inhalation exposure scenarios. The HIARC also evaluated available studies to determine if there is a special sensitivity for infants and children. In a subsequent HIARC meeting on 12/18/00 following the FIFRA Scientific Advisory Panel [SAP] report on a preliminary hazard and dose-response assessment on atrazine [6/27/00] and the Risk Assessment Review Committee [RARC] review of the preliminary Human Health Assessment for the Reregistration Eligibility Decision [RED] on atrazine, the HIARC revisited the recommendations and conclusions drawn at the 5/4/00 meeting.

Atrazine has low acute toxicity. Toxicity categories are III or IV by all routes of exposures (oral, dermal, inhalation, ocular), and atrazine is not a skin sensitizer.

Atrazine has a low toxicity by the dermal route as indicated by the acute dermal toxicity study and by a 21-day dermal toxicity study using New Zealand white rabbits. This 21 day study found a LOAEL at the limit dose of 1,000 mg/kg/day and had a NOAEL at 100 mg/kg/day.

Numerous mutagenicity studies, both submitted guideline studies and open literature studies, are available. The weight of evidence does not support a mutagenic potential or concern for Atrazine.

The primary effects seen in rats following chronic exposure to atrazine are neuroendocrine perturbations and mammary and pituitary gland tumors.

The neuroendocrine disruptions include alterations in hypothalamic neurotransmitter and neuropeptide levels which have been described by EPA researchers at EPA's National Health and Environmental Effects Research Labs (NHEERL). Decreases in serum levels of pituitary luteinizing hormone (LH), presumably a consequence of these hypothalamic alterations, have been reported both by NHEERL researchers and in registrant-submitted studies. In the Sprague-Dawley rat, decreased LH levels would be expected to result in estrous cycle disruptions and, concurrently, increased and/or prolonged exposure of endocrine-responsive tissues to certain hormones such as estrogens and prolactin. Such alterations following atrazine exposure in the rat have been reported by both NHEERL researchers and registrant-submitted studies. NHEERL has also found that Atrazine can suppress pituitary prolactin release by suppressing hypothalamic dopamine release after short-term dosing.

This cascade of events described in the previous paragraph - the earliest clearly identified event being neurotransmitter alterations in the hypothalamus and the terminal alteration being increased/prolonged exposure of endocrine-responsive tissues to estrogens and prolactin - have been hypothesized to constitute a mode of action (MOA) relating to the tumor findings seen in chronic bioassays using the Sprague-Dawley (SD) rat. Registrant-submitted studies, both guideline studies and non-guideline studies, have demonstrated that atrazine exposure is associated with increased incidences and/or earlier onset of mammary and pituitary gland tumors in female SD rats. Registrant-submitted chronic bioassays, both guideline and non-guideline studies, using the CD-1 mouse and F-344 rat were negative for carcinogenicity in both sexes.

The FIFRA Science Advisory Panel (SAP) met on June 27th to 29th, 2000 to discuss data pertaining to the atrazine-associated cancer in rats and these neuroendocrine alterations, as well as its reproductive and developmental effects. Hypothalamic neuronal - GnRH pulse modulation of pituitary releases of LH is a central driver of ovulation in the SD female rat, and atrazine is essentially accelerating the aging process of the CNS control of ovulation. This leads to a constant state of estrus (anovulation) and prolonged exposure to estrogen and prolactin. Although, there are certain similarities in the control of the hypothalamic-pituitary- ovarian axis between humans and rats in that the hypothalamus can play a key regulatory role in primates, there are fundamental differences. Although atrazine might cause adverse effects on hypothalamic-pituitary function in humans, the hormonal environment conducive to tumor development (i.e., elevated or prolonged exposure to estrogen or prolactin) that is found in SD rats can not be established in humans. The SAP concluded that the neuroendocrine alterations in the SD rat constitute the mode of action for atrazine-associated carcinogenicity. The Health Effects Division Cancer Peer Review Committee (CARC, 10/19/00) has concurred with the SAP and has concluded that these neuroendocrine events constitute a mode of action that is operative in the SD female rat, which is susceptible to these effects due its mode of reproductive senescence (SD females undergo a different mode of reproductive senescence than do F-344 rats, in which chronic bioassays were negative for cancer). CARC has classified atrazine as a "Not Likely To Be Carcinogenic To Humans".

These same previously described neuroendocrine alterations, which constitute the MOA for cancer, could be expected to be associated with reproductive or developmental effects. Studies from EPA's NHEERL labs have demonstrated reproductive/developmental effects including prostate inflammation in male rats whose mothers' prolactin was suppressed due to treatment with atrazine while lactating, as well as delay of puberty in both male and female rats exposed prior to pubertal onset.

Though special studies conducted at EPA labs, described in the previous paragraph, indicate developmental/reproductive toxicity, guideline studies have, for the most part, not indicated developmental/reproductive toxicity. There was no evidence of increased sensitivity/susceptibility in two rat and one rabbit developmental toxicity studies using atrazine or in a rat developmental toxicity study using deisopropyl atrazine or a rat developmental toxicity study using deethyl atrazine (these compounds are metabolites of atrazine). Although there was also no evidence of increased sensitivity/susceptibility in a two-generation study with atrazine, it should be noted that the available study was performed under the old protocol, and the new two-generation reproduction test protocol has parameters that are sensitive to endocrine disruption of reproductive development and function. The one exception was that there was evidence of increased sensitivity/susceptibility in a developmental toxicity study using diaminochlorotriazine (DACT), the terminal chloro-metabolite of atrazine. In this study the maternal LOAEL/NOAEL was 75/25 mg/kg/day based on decreased body weight gain during dosing. The developmental LOAEL/NOAEL in this study was 25/2.5 mg/kg/day based on increases in incidence of incompletely ossified parietals, interparietals and unossified hyoids.

Based on the results from the DACT developmental toxicity study and the data from the NHEERL studies, the HED Hazard Identification and Review Committee (HIARC) at a meeting held 5/4/2000, recommended that a two-generation reproduction study with DACT using the Series 870.3800 guidelines be performed. HIARC also recommenced that additional measures examining effects of the type seen in the NHEERL studies be incorporated.

Atrazine is not readily absorbed through dermal exposure. Based on both a rat and a human dermal absorption study, HIARC recommended a dermal absorption factor of 6% to be used for risk assessment.

The only outstanding datagap is a Series 870.3800 two-generation reproduction study for the chloro-metabolite DACT, described above. Though not required or considered a datagap, HIARC also recommenced (5/4/00 meeting) that studies investigating potential reproductive/developmental effects that may be related to the neuroendocrine alterations described above, be performed.

2.0 REQUIREMENTS

The requirements (CFR 158.340) for food use of atrazine are in Table 1.

Table 1.

Test	Technical	
	Required	**Satisfied**
870.1100 Acute Oral Toxicity	yes	yes
870.1200 Acute Dermal Toxicity	yes	yes
870.1300 Acute Inhalation Toxicity	yes	yes
870.2400 Primary Eye Irritation	yes	yes
870.2500 Primary Dermal Irritation	yes	yes
870.2600 Dermal Sensitization	yes	yes
870.3100 Oral Subchronic (Rodent)	yes	yes
870.3150 Oral Subchronic (Non-Rodent)	yes	yes[a]
870.3200 21-Day Dermal	yes	yes
870.3250 90-Day Dermal	no	-
870.3465 90-Day Inhalation	no	-
870.3700a Developmental Toxicity (Rodent)	yes	yes
870.3700b Developmental Toxicity (Non-rodent) . . .	yes	yes
870.3800 2-Generation Reproduction	yes	no[b]
870.4100a Chronic Toxicity (Rodent)	yes	yes
870.4100b Chronic Toxicity (Non-rodent)	yes	yes
870.4200a Oncogenicity (Rat)	yes	yes
870.4200b Oncogenicity (Mouse)	yes	yes
870.4300 Chronic/Oncogenicity	yes	yes
870.5100 Mutagenicity—Gene Mutation - bacterial		
870.5375 Mutagenicity—Structural Chromosomal	yes	yes
Aberrations		
870.5550 Mutagenicity—Other Genotoxic Effects	yes	yes
	yes	yes

Test	Technical	
	Required	**Satisfied**
870.6100a Acute Delayed Neurotox. (Hen)	no	-
870.6100b 90-Day Neurotoxicity Hen)	no	-
870.6200a Acute Neurotox. Screening Battery (Rat)		
870.6200b 90 Day Neuro. Screening Battery (Rat) .	no	no
870.6300 Develop. Neuro	no	no
	no	no
870.7485 General Metabolism	yes	yes
870.7600 Dermal Penetration	yes	yes

a Requirement waived because a chronic study in the non-rodent (*i.e.* - dog) is available
b Although an acceptable multigeneration study is available for atrazine, HIARC has requested a new study [OPPTS Series 870.3800 guideline] using the atrazine metabolite diaminochlorotriazine (DACT).

3.0 DATA GAPS

Status of previous datagaps

The previous Toxicology RED chapter for atrazine (TXR 006937) completed in 1988, identified several data gaps. Table two displays those data gaps and the studies that have been submitted to fill these gaps. All datagaps described in the previous Toxicology RED chapter have been fulfilled.

Table 2: Data Gaps Listed in 1988 RED Document and Studies That Have Been Submitted to Fill these Data Gaps.

1988 Data Gap	**Results of Submitted Study**	**MRID No(s).**
81-3, Acute Inhalation	Study Acceptable-Data Gap Filled	42089901, 43016502
82-2, Subchronic Dermal	Study Acceptable-Data Gap Filled	42089902
84-2, Mutagenicity - a. Dominant Lethal Assay b. Direct DNA Damage	Studies Acceptable-Data Gaps Filled	426370003 and 42547105

Current datagaps

There are no datagaps for atrazine. However, the HIARC has required that a OPPTS Series 870.3800 Reproduction and Fertility Effects study be performed with the atrazine metabolite diaminochlorotriazine (DACT) - a mammalian metabolite of both atrazine and simazine. Otherwise there are no datagaps for atrazine, or the atrazine metabolites listed in this document, according to the OPPTS Series 870 Guideline requirements.

4.0 HAZARD ASSESSMENT

4.1 Acute Toxicity

Adequacy of data base for acute toxicity: The data base for acute toxicity is considered complete. No additional studies are required at this time.

The acute toxicity data on atrazine technical is summarized below in Table 3.

Table 3: Acute Toxicity Data of Technical Atrazine

Guideline No.	Test	Results	Toxicity Category
870.1000	Oral LD_{50} - rat	LD_{50} > 1,869 mg/kg (M&F combined)	III
870.1200	Dermal LD_{50} - rat	LD_{50} > 2,000 mg/kg (M&F combined)	III
870.1300	Inhalation LC_{50} - rat	LC_{50} > 5.8 mg/L (M&F combined)	IV
870.1400	Eye Irritation - rabbit	PIS[a] = 0.0/110	IV
870.1500	Dermal Irritation - rabbit	PIS[a] = 0.2/8.0	IV
870.1600	Dermal Sensitization - guinea pig	Non-sensitizing	---
870.6100	Acute Delayed Neurotoxicity	Not Required	---

a PIS=Primary Irritation Score

870.1100. Acute oral toxicity - rats

A pair of acute LD_{50} studies in the rat using technical grade atrazine have shown the LD_{50} to be: 1,869 mg/kg (Accession No. 230303; MRID 00024706) and 2,850 mg/kg (MRID 00027097). Numbers in all these studies are for both sexes combined. The toxicity category for acute oral exposure is at least III.

870.1200. Acute dermal toxicity - rat and rabbit

Two dermal LD_{50} studies using the rat resulted in LD_{50}s of >2,000 mg/kg and > 3,100 mg/kg for both sexes combined (Accession Nos. 00027097 and 230303; MRID 00024708). A study using rabbits, the preferred species in the OPPTS Series 870 guidelines, resulted in an LD_{50} of 7,550 mg/kg for both sexes combined (Accession No. 231466). Atrazine is at least toxicity category III for dermal exposure.

870.1300. Acute inhalation toxicity - rats

The inhalation LC_{50} was found to be 5.82 mg/L (MRIDs 42089901 and 43016502). This exceeds the limit dose of 5.0 mg/L and places atrazine in toxicity category IV.

870.2400. Primary ocular irritation - rabbits

Atrazine tested negative for primary eye irritation and has been placed in toxicity category IV for this endpoint (Accession No. 230303; MRID 00024709).

870.2500. Primary dermal irritation - rats

Atrazine tested negative for primary skin irritation, and has been placed in toxicity category IV for this endpoint (Accession No. 230303; MRID 00024710).

870.2600. Dermal sensitization - guinea pigs

Atrazine has been shown to be non-sensitizing in dermal sensitization studies (MRID 00105131).

870.6100. Acute and delayed type neurotoxicity of organophosphorus substances

Acute neurotoxicity studies have not been performed on atrazine, and none are required.

4.2 Subchronic Toxicity

Adequacy of data base for subchronic toxicity: The data base for subchronic toxicity is considered complete. No additional studies are required at this time.

870.3100 90-Day Oral Toxicity - Rat

In a subchronic oral toxicity study (MRID 44723701) Atrazine Technical (purity 97.1 %) was administered in the diet to Tif: RAIf (SPF), RII/1 x RII/2 hybrids, (Sprague-Dawley derived) rats 10/sex/dose at dose levels of 0, 10, 50, or 500 ppm for 92 days. These doses were equivalent to 0, 0.6, 3.3, and 34.0 mg/kg/day for males, and 0, 0.659, 3.35, and 35.3 mg/kg/day for females. Rats were treated for 13 weeks, then sacrificed (S1). Additional recovery groups of 10 rats/sex/dose were fed 0 or 500 ppm for 13 weeks, remained untreated for 4 weeks, and were then sacrificed (S2).

One female in the control group died during the study. No treatment-related clinical effects were noted. Treatment with atrazine technical resulted in significant ($p<0.01$) decreases in body weight in high dose males and females compared to controls. Body weight losses were partially regained during the recovery period. Hematological and clinical chemistry parameters showed no significant treatment related effects. Decreased liver and kidney weights in high dose males occurred as a result of the marked body weight loss. There were no treatment-related effects on gross pathology or ophthalmology. Hemosiderin pigment was found in the spleen at an increased incidence and severity in high dose animals of both sexes, at the end of treatment and recovery periods.

As tested in this study, the LOAEL for Atrazine is 34.5 mg/kg/day based on decreased body weight. The NOAEL is 3.3 mg/kg/day.

This subchronic toxicity study is classified as **Acceptable /Guideline** and satisfies the guideline requirement for a subchronic oral study (OPPTS 870.3100) in rats.

870.3150 90-Day Oral Toxicity - Dog

Study has been waived because an acceptable chronic study is available.

870.3200 21-Day Dermal Toxicity - Rabbit

A 21 day dermal study (MRID 42089902) in New Zealand White rabbits was conducted. Rabbits, 5 rabbits/sex/dose, were administered atrazine (97.6%) 6 hrs per day for 25 days at concentrations of 0, 10, 100 or 1000 mg/kg/day.

Systemic toxicity was observed at the high dose tested (HDT) and consisted of reductions in food consumption(decreased 28% for males and 31% for females at day 21 *vs* controls) and mean body weight(12% at day 25 *vs* controls for males and 19% for females). Percent body weight gain was decreased(-4.4% *vs* +9.5 at day 21 for males and -11% *vs* +8% for females). Compared to controls, relative and absolute spleen weights in both sexes were increased (males had an 81% absolute increase and 75% increase relative to body weight; females had an 86% increase absolutely and 94% relative to body weight).

Additional findings were reduced red blood cell and hematocrits counts (each decreased by 12% *vs* controls), and increased cholesterol and triglyceride levels (increased by 101 and 55% *vs* controls) in the highest dose tested [HDT] females and reduced total protein and chloride levels (decreased by 7 and 5% *vs* controls) in HDT males. Dermal findings were: limited- to- slight acanthosis for both sexes at all dose levels; minimal-to-moderate acanthosis in 3 HDT females (compared to zero control females having this finding); and 3 cases of focal subacute inflammation in the treated skin of HDT females (compared to zero cases of this in the controls). The NOAEL is 100 mg/kg/day and the LOAEL at 1000 mg/kg/day based on reductions in food consumption, mean body weight, body weight gain and increases in spleen weight.

The LOAEL is 1000 mg/kg/day based on reduced food consumption, mean body weight and body weight gain, increased relative and absolute spleen weights, and reduced red blood cell counts and hematocrits. The NOAEL is 100 mg/kg/day.

This study is classified **Acceptable-Guideline** and satisfies the 870.3200 series guidelines for a 21 day dermal toxicity study in the rabbit.

4.3 Prenatal Developmental Toxicity

Adequacy of data base for Prenatal Developmental Toxicity: The data base for prenatal developmental toxicity is considered complete. No additional studies are required at this time.

870.3700 Prenatal Developmental Toxicity Study - Rat (1989 study)

In a developmental toxicity study (MRID 41065201) atrazine (97.6%) was administered by gavage to 104 mated female Sprague-Dawley rats, 26/dose, at dose levels of 0, 5 (LDT), 25 (MDT), 100 (HDT) mg/kg/day from days 6 through 15 of gestation.

Maternal toxicity findings were almost exclusively confined to the high dose group. Compared to controls high dose dams displayed: reduced food consumption (decreased 13%, $p \leq 0.5$); reduced total body weight gain (reduced 18% during dosing period, $p \leq 0.5$); reduced corrected (minus uterine weight) weight gain (reduced 20% for entire gestation, $p \leq 0.5$); and increased alopecia 1/26 controls vs 5/26 high dose). One high dose animal died on gestation day 20 and salivation was noted as an observation in 18/26 high dose animals. The only observations seen outside the high dose group were: an abortion from one of the mid-dose animals on gestation day 19; a fluid-filled hollow right kidney in a mid-dose animal; and hollow discolored kidneys in a low dose animal.

The maternal LOAEL is 100 mg/kg/day based on reduced body weight gain and food consumption. The maternal NOAEL is 25 mg/kg/day.

The few malformations seen upon external examination of the fetuses were seen only in the control groups and clearly could not be compound related. Likewise, there was no increased incidence of visceral malformation in dosed groups *vs* control groups. There were no skeletal malformations observed but there was an increased incidence of incomplete ossification of various bones in the highest dose tested [HDT]. Hyoids (control fetal incidence of 11% *vs* 21.7% HDT), occipitals (7.7% *vs* 21.1%) and parietals (2.2% *vs* 8.4%) showed incomplete ossification. There was also an increased incidence ($p \leq 0.05$) of incomplete ossification of the interparietals in all dose groups compared to controls.

Fetal body weight, number of resorptions and implantations, and live fetuses/litter were not significantly affected by atrazine treatment. Exposure of gravid Sprague-Dawley rats to atrazine under the conditions described in this study seemed to have few embryo/fetotoxic effects.

The developmental LOAEL is 100 mg/kg/day based on increased incidence of delayed ossification of skull bones. The developmental NOAEL is 25 mg/kg/day.

The developmental toxicity study (MRID 41065201) in the rat is classified **Acceptable-Guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700) in the rat.

870.3700 Prenatal Developmental toxicity - Rat (1984 study)

In a developmental toxicity study (MRID 00143008 and 40566302) atrazine (96.7%) was administered to 104 Charles River CD rats 27/dose by gastric intubation at dose levels of 0, 10, 70, or 700 mg/kg/day from days 6 through 15 of gestation.

Mortality was very high for the 700 mg/kg/day animals in this study. All but 6 of the 27 females in this group died during the gestation period. Other statistically significant findings in this group included: salivation; oral and nasal discharge; ptosis; swollen abdomens; blood on the vulva; enlarged stomachs and adrenal; and discolored lungs. Body weight gains were statistically significantly reduced throughout most of the gestation period. Significant reductions in food consumption were observed. Although mortality was quite high in the high dose group, pregnancy rates were comparable to controls (85.2% for controls *vs* 96.3% high dose) and the numbers of live fetuses at c-section was comparable to controls (mean of 12.7 per litter for controls *vs* 13.4 for the high dose).

There were few findings in either the low or mid dose animals. Alopecia was observed in mid dose animals, but was not considered to be biologically significant. Body weight gain in the mid dose group was significantly reduced for the first 5 days of compound exposure (gestation days 6-10). Food consumption in the intermediate group was reduced, but only at day 17 of gestation. Low dose body weight gain was increased on days 6-10 of gestation and food consumption was increased on gestation day 9.

The maternal LOAEL is 70 mg/kg/day, based on reduced body weight gain. The maternal NOAEL is 10 mg/kg/day.

Fetal weights were statistically significantly reduced in the high dose group. Skeletal examinations were not conducted in the high dose group due to the extremely low fetal weights. Visceral and external examinations were conducted, though, and no group, including the high dose group, displayed any findings significantly different from control values. Skeletal anomalies, however, were observed in the mid dose group.

The developmental LOAEL was found to be 70 mg/kg/day, based on delayed or no ossification at several sites. The developmental NOAEL is 10 mg/kg/day.

The developmental toxicity study in the rat is classified **Acceptable-Guideline** and does satisfy the 870.3700 guideline requirement for a developmental toxicity study in rats.

870.3700 Prenatal Developmental Toxicity Study - Rabbit

In a developmental toxicity study (Accession No. 254979 and MRIDs 00143006, 40566301) atrazine (96.3%) was administered by gavage to 76 mated female New Zealand White rabbits, 19/dose, at dose levels of 0, 1, 5, or 75 mg/kg/day, from days 7 through 19 of gestation.

Clinical signs seen in 75 mg/kg/day (highest dose tested; HDT) animals that were considered to be related to compound treatment were stool changes (none, little or soft stool; 9/19 controls *vs* 19/19 HDT), and the appearance of blood in the cage or on the vulva (0/19 controls *vs* 4/19 HDT). Body weight gain was reduced in high dose dams and, at many time points, body weight was below day zero values. At gestation days 14, 19, 21 and 25, mean maternal body weights were 12%, 19%, 18%, and 10% below control values($p \leq 0.01$ for all four of these time points).

High dose animals displayed significantly reduced food consumption during treatment. During gestation days 12 to 17 the HDT average feed consumption was only 1-6 grams of feed per animal per day compared to 175-182 grams for the controls.

The mid and low dose groups had no alterations that could be attributed to atrazine exposure.

The maternal toxicity LOAEL is 75 mg/kg/day based on decreased body weight, food consumption and increased incidence of clinical signs. The maternal toxicity NOAEL is 5 mg/kg/day.

Fetal effects were: increased resorptions - mean of 1.3/dam in controls *vs* 4.8/dam in HDT - ($p \leq 0.01$), reduced live fetuses per litter - mean of 8.8/dam in controls *vs* 5.9/dam in HDT - ($p \leq 0.05$), and increased delayed ossification of appendicular elements were observed in the high dose group.

The low and intermediate groups had no fetal findings that could be attributed to compound exposure.

The findings in the high dose group were determined to be secondary to maternal toxicity and thus the LOAEL and NOAEL for embryo/fetotoxicity match the maternal LOAEL and NOEL.

The developmental toxicity LOAEL is 75 mg/kg/day based on reduced litter size, increased resorptions and delayed ossification. The developmental toxicity NOAEL is 5 mg/kg/day

The study is considered **Acceptable-Guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700) in rabbit.

4.4 Reproductive Toxicity

Adequacy of data base for Reproductive Toxicity: Although there is an acceptable multi-generation reproduction study available for atrazine, an additional study is being requested using the diamino-chlorotriazine (DACT) metabolite (see section 4.10.1 below for further discussion on this).

870.3800 Reproduction and Fertility Effects - Rat

In a 2-generation reproduction study (MRID 40431303) atrazine, (purity not specified but said to be technical grade) was administered to 240 Charles River (CRCD, VAF/PLUS) rats 30/sex/dose in the diet at dose levels of 0, 10, 50, and 500 ppm.

There was very little variation in test article consumption between generations; the F_0 and F_1 males had similar test article consumption during the 70-day premating period as did the F_0 and F_1 females. The average values for the two generations are 0, 0.75, 3.78, 39.0 mg/kg/day for males and 0, 0.86, 3.70, 42.8 mg/kg/day for females. Test article consumption for the F_0 and F_1 generation females during their gestation period did not vary greatly between generation. Mean compound consumption for both generations were 0, 0.66, 3.33 and 35.43 mg/kg/day.

Parental body weights, body weight gain, and food consumption were statistically significantly reduced at the 500 ppm dose (highest dose tested; HDT) in both sexes and both generations throughout the study. Compared to controls, body weights for F_0 HDT males and females at 70 days into the study were decreased by 12% and 15%, respectively while F_1 body weight for the same time period was decreased by 15% and 13% for males and females, respectively. The only other parental effect which may have been treatment related was a slight, but statistically significant, increase in relative testes weight which occurred in both generations of the HDT. There did not appear to be any reproductive effects from compound exposure. Measured reproductive parameters from both generations did not appear to be altered in a dose-related manner. NOTE: Although there was also no evidence of increased sensitivity/susceptibility in this two-generation reproduction study with atrazine, it should be noted that the available study was performed under the old protocol, and the new two-generation reproduction test protocol has parameters that are sensitive to endocrine disruption of reproductive development and function.

The LOAEL is 500 ppm (39 mg/kg/day in males, 42.8 mg/kg/day in females) based on decreased body weights, body weight gains and food consumption. The NOAEL is 50 ppm(3.78 mg/kg/day in males, 3.7 mg/kg/day in females)

The developmental toxicity LOAEL is 39 mg/kg/day in males, 42.8 mg/kg/day in females, based on decreased body weights in both generations of males at postnatal day 21. The developmental toxicity NOAEL is 3.78 mg/kg/day in males and 3.7 mg/kg/day in females.

This study is classified **Acceptable-Guideline** and satisfies the guideline requirement for a 2-generation reproductive study (OPPTS 870.3800) in the rat.

4.5 Chronic Toxicity and Carcinogenicity

Adequacy of data base for chronic toxicity: The data base for chronic toxicity is considered complete. No additional studies are required at this time.

870.4100 (870.4300) Combined Chronic Toxicity/Carcinogenicity – Rat (Sprague-Dawley)

In a combined 2-year feeding/oncogenicity study (MRID 40629302) atrazine, 95.9% +/-2.3% was administered to Sprague-Dawley rats, 20/sex/dose, in the diet, at dose levels of 0, 10, 70, 500, and 1000 ppm, (0, 0.5, 3.5 25, 50 mg/kg/day) for 2 years.

Toxicologic alteration appeared to be confined to the 500 and 1000 ppm dose groups.

25 mg/kg/day: Both male and female rats showed reductions in body weight gain, and food consumption. Body weight for males and females at 104 weeks was 8% and 19% below 104 week control values. Food consumption at 104 weeks was not significantly below control, but at weeks 1, 13 and 26 for males and week 1 for females, food consumption was statistically- significantly reduced. Food consumption was reduced 11% at week 1, 11% at week 13 and 9% at week 26 in males and 8% at week 1 in females. Males displayed no unusual histopathology findings in this group, but females had statistically-significant increases of myeloid hyperplasia in the bone marrow of the femur (25 control incidences *vs* 38) and sternum (21 *vs* 33), and splenic extra medullary hematopoiesis (12 *vs* 22).

50 mg/kg/day: Both sexes showed reductions in body weight gain (19% *vs* controls at 104 weeks for males, 27% at 104 weeks for females)and food consumption (reduced *vs* controls 20%, 16%, 14% and 11% at weeks 1, 13, 26 and 52 for males and 18%, 8%,and 7% at weeks 1, 13, and 26 for females). Female survival was 50% at 104 weeks in controls and 26% in 1000 ppm animals. Interestingly, survival was increased in 1000 ppm males - 44% control survival *vs* 67% 1000 ppm at 104 weeks. Altered hematology and clinical chemistry findings were noted in females and included: decreased hemoglobin concentration; hematocrit; RBC; and serum glucose. Males did not display these alterations but did display decreased serum triglyceride levels throughout the course of the study. Organ-to-body weight ratios were decreased in high dose animals which may have been the result of decreases in body weight gain. Histopathology findings in females dosed 1000 ppm consisted of statistically-significant increases in incidences of (% increase *vs* controls in parenthesis): retinal degeneration (83%); centrilobular necrosis in the liver (300%); degeneration of the rectus-femoris muscle (160%); transitional epithelial hyperplasia in the bladder (150%) and the kidney (82%); splenic extra medullary hematopoiesis (133%); and myeloid hyperplasia in both the femur (108%) and sternum bone marrow (119%). Histopathology findings in males dosed 1000 ppm consisted of statistically-significant increases in incidences of: degeneration rectus femoris muscle (366%); prostate epithelial hyperplasia (141%); calculi in the kidney pelvis(106%); and acinar hyperplasia of the mammary gland (200%).

A carcinogenicity study run concurrently with the toxicity study determined that there was a dose-related increase in mammary adenocarcinomas (p value for the trend < 0.00005) in 70, 500 and 1000 ppm females. Adenocarcinomas incidences were: controls -15/66; 70 ppm - 26/68; 500 ppm - 27/65; 1000 ppm - 35/64.

The dose response curve in this study appeared to be adequate as the low doses (10 and 70 ppm) showed few toxic effects; the mid-dose level showed some toxic effects (reduced body weight gain and food consumption); while the high dose showed the same effects as the mid dose plus hematology/clinical chemistry and histopathology findings.

The LOAEL for non-cancer effects is 500 ppm (25 mg/kg/day), based on reduced body weight gain and food consumption. The NOAEL is 70 ppm (3.5 mg/kg/day). Mammary tumors [adenocarcinomas] were observed at dose levels of 70 ppm (3.5 mg/kg/day) and above.

This chronic toxicity study in the rat is **Acceptable-Guideline** and satisfies the guideline requirement for a combined chronic oral/carcinogenicity study in the rat (870.4300).

NOTE: Please refer to Hazard Assessment and Review of Available Studies [Part B] of the May 22, 2000 PRELIMINARY DRAFT Hazard and Dose-Response Assessment and Characterization- ATRAZINE, presented to the FIFRA Scientific Advisory Panel [SAP] on June 27-29, 2000 for a summary and details of this study as well as the other long-tern studies in SD rats (see http://www.epa.gov/scipoly/sap/2000/june27/finalparta_atz.pdf)**.**

870.4100 (870.4300) Combined Chronic Toxicity/Carcinogenicity – Rat (F-344)

In a chronic toxicity/carcinogenicity toxicity study (MRID 42227001), atrazine was administered to 600 Fischer- 344 rats, 60/sex/dose in the diet at dose levels of 0, 10, 70, 200, 400 ppm (0, 0.49, 3.43, 9.87 and 20.17 mg/kg/day for males and 0, 0.61, 4.35, 12.71, and 26.18 mg/kg/day for females) for 104 weeks.

Administration of atrazine did not affect animal survival nor, with the exception of thinness in the high-dose group, were any clinical signs apparent. Body weight and body weight gain were significantly reduced in 200 and 400 ppm exposed animals of both sexes throughout most of the study. Food consumption in the high dose group males was significantly reduced throughout the study, and significantly reduced in high dose females for the first 13 weeks of exposure. No findings were seen at necropsy which could be attributed to compound exposure. There were no significant increases in either neoplastic or non-neoplastic findings which could be attributed to compound exposure.

The LOAEL is 3.9 mg/kg/day based on decreased body weight gain. The NOAEL is 0.55 mg/kg/day.

At the doses tested, there was no treatment-related increase in tumor incidence when compared to controls. Dosing was considered adequate based on decreased body weight gain.

This carcinogenicity study in the Fischer 344 rat is **Acceptable-Guideline** and does satisfy the guideline requirement for a carcinogenicity study (83-2a) in the rat.

NOTE: Please refer to Hazard Assessment and Review of Available Studies [Part B] of the May 22, 2000 PRELIMINARY DRAFT Hazard and Dose-Response Assessment and Characterization - ATRAZINE, presented to the FIFRA Scientific Advisory Panel [SAP] on June 27-29, 2000 for a summary and details of this study as well as the other long-tern studies in F-344 rats (see http://www.epa.gov/scipoly/sap/2000/june27/finalparta_atz.pdf **).**

870.4100b Chronic Toxicity - Dog

In a chronic toxicity study (MRID 40431301) atrazine, 97%, was administered to 40 Beagle dogs, 4/sex in the low and intermediate dose groups and 6/sex in the control and high dose groups, in the diet at dose levels of 0, 15 (LDT), 150 (MDT) and 1000 (HDT) ppm for 52 weeks. The mean daily dose 0, 0.48, 4.97, 33.65 mg/kg for males. Female doses were the same for the low and mid dose and only slightly different in the high dose - 33.80 mg/kg/day in the high dose group.

Percent increases in body weight during the course of the study were as follows: male: 46.6% in control; 52.5% LDT; 36.8% MDT and 29.5% for the HDT; female: controls; 44.4% for LDT; 35.9% MDT and 21.5% HDT. Only for the males at the HDT was this increase statistically significant (SS) compared to the control increase.

Although only statistically-significant for the first quarter, HDT males and females showed reduced food consumption throughout the course of the study. By studies' end, mean food consumption was decreased 22% in males and 20% in females.

High dose animals recorded statistically-significant changes in organ weights for both sexes compared to controls. Absolute heart weight was decreased in females by 14% and male liver weight relative to body weight was increased by 47%.

The major treatment-related findings in this study were cardiac findings. EKG alterations such as tachycardia and increased P wave amplitude (P-II), were observed at many timepoints during the study in HDT animals. Mean heart rate was increased 59% ($p \leq 0.05$) and P-II was decreased 78% ($p \leq 0.01$) on day 361 in HDT males compared to controls. Female heart rates at the same time point were increased

46% which was not statistically significant, but P-II was decreased 71% which was significant at $p \leq 0.01$. Gross pathology findings were also seen in the heart and were mostly concentrated in the HDT group. Minimal atrial dilation seen in 3/6 males and 2/6 females was the only cardiac finding seen at necropsy in the control animals. The HDT group, on the other hand, displayed moderate to severe atrial dilation in 4/5 males and 5/5 females who survived until scheduled sacrifice. Two of 5 HDT males who survived until scheduled sacrifice also displayed enlarged, soft hearts and 1/5 had fluid in the pericardium.

Histopathology findings were also seen in the heart. Three of 6 HDT males and 6/6 HDT females were found to have cardiac myolysis while no control animals of either sex had myolysis. Focal atrophy of the heart was seen in 2/6 HDT males and 5/6 HDT females while no controls of either sex displayed this finding.

Three animals, two high dose and one mid-dose, were sacrificed in moribund condition during the course of the study. The two high dose sacrifices may have been due to compound-related cardiac dysfunction.

The LOAEL for both sexes is 1000 ppm (33.65 mg/kg/day for males and 33.8 for females), based on cardiac effects. The NOAEL is 150 (4.97 mg/kg/day) for both sexes.

The major finding of this study was cardiopathology. A broad array of cardiac dysfunctions seen in the high dose group such as: electrocardiography alterations; the appearance of both microscopic and macroscopic cardiac abnormalities, were determined to be due to atrazine exposure. The wide variety of effects seen, as well as possible compound-related animal deaths, indicate the severity of the cardiac effects.

This chronic toxicity study is **Acceptable-Guideline** and satisfies the guideline requirement (870.4100) for a chronic oral toxicity study in dog.

870.4200 Carcinogenicity Study - rat

See above under "870.4100 (870.4300) Chronic Toxicity – rat" for executive summary.

870.4200 Carcinogenicity - mouse

In an carcinogenicity study (MRID 40431302), atrazine, (purity not given) was administered to CD-1 mice, 59-60/sex/dose, in the diet at dose levels of 0, 10, 300, 1500 and 3000 ppm (male/female mean daily dose 0/0, 1.4/1.6, 38.4/47.9, 194.0/246.9, 385.7/482.7 mg/kg/day) for 91 weeks. Female mice in the 300, 1500 and 3000 ppm groups received a daily atrazine dose about 25% higher than their counterpart males.

No dose-related increases in neoplasms were observed. The dose response curve seemed adequate since toxic effects, such as a decrease in mean body weight of both sexes and an increase in cardiac thrombi in the females, are seen at both 1500 and 3000 ppm, while no dose-related toxic effects are seen at 10 and 300 ppm. In addition to the toxic effects just mentioned, the 3000 ppm animals of both sexes also displayed decreases in food consumption and decreases in RBC, hematocrit, and hemoglobin concentration. Female mice, but not males, at 3000 ppm showed decreased mean group brain and kidney weights and decreased percentages of neutrophils and lymphocytes. There was also an increase in mortality ($p < 0.05$) in 3000 ppm females, but not males, with only 25% of the females surviving *vs* 39-43% of the females surviving in the other female dose groups.

The cardiac thrombi found at both 1500 and 3000 ppm may have contributed to unscheduled female deaths during the course of the study. The incidence of unscheduled death in mice with cardiac thrombi is statistically significantly different from the incidence of unscheduled death in mice from control groups. The occurrence of cardiac thrombi is considered a severe effect.

The LOAEL is 222.0 mg/kg/day, based on decreased body weight gain in both sexes and increased cardiac thrombi in the females. The NOAEL is 43 mg/kg/day.
At the doses tested, there was not a treatment-related increase in tumor incidence when compared to controls. Dosing was considered adequate due to the occurrence of decreased body weight gain and cardiac thrombi.

This carcinogenicity study in the mouse is **Acceptable-Guideline,** and does satisfy the guideline requirement for a carcinogenicity study in the mouse (870. 4200).

4.6 Mutagenicity

Adequacy of data base for Mutagenicity: The data base for Mutagenicity is considered adequate based on current and pre -1991 mutagenicity guidelines.

Numerous open literature mutagenicity studies are available for evaluation. The entire mutagenicity database for atrazine (including these open literature studies) has been reviewed by CARC (October 13, 1999; April 12, 2000; May 22, 2000 {http://www.epa.gov/scipoly/sap/ #june27}, October 19, 2000) and by the Science Advisory Panel (June, 28 - 30, 2000) and both panels stated that the available evidence did not indicate a mutagenic effect of atrazine exposure.

870.5100 Bacterial reverse mutation test

In a reverse gene mutation assay in bacteria (MRID 40246601), strains TA 98, 100, 1535 and 1537 of S. typhimurium were exposed to atrazine (98.2% a.i.), in dimethylsufoxide, at concentrations of 0, 20, 78, 313, 1250, and 5000 µg/plate. Test were conducted in the presence and absence of mammalian metabolic activation S9 fraction of Tif:RAIf rats treated with Aroclor 1254.

Atrazine was tested up to the limit concentration, 5000 µg/plate. The positive controls did induce the appropriate responses in the corresponding strains. **There was no evidence of inducedmutant colonies over background.**

This study is classified as **Acceptable-Guideline**. It does satisfy the requirement for OPPTS Test Guideline 870.5100 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

870.5385 Mammalian Bone Marrow Chromosome Aberration Test

A mouse bone marrow micronucleus test was conducted using Tif:MAGF mice (MRID

40722301). The test consisted of two parts. The first portion consisted of 24 male and 24 female mice being dosed with 2250 mg/kg atrazine (98.2% a.i.). Eight animals of each sex were then sacrificed at 16, 24 or 48 hours following treatment. The second portion of the study 24 mice, 8/sex/dose, were treated with atrazine (98.2% a.i.) at doses of 562.5, 1175, 2250 mg/kg. Bone marrow cells were harvested at 24 hours post-treatment. The vehicle in both portions of the study was carboxymethyl cellulose. Exposure in both portions of the study was accomplished by a single gastric intubation.

There were no signs of toxicity in either portion of the study. Atrazine was tested at an adequate doses being that these were doses that induced death in mice. The positive control induced the appropriate response. **There was not a significant increase in the frequency of micro nucleated polychromatic erythrocytes in bone marrow after any treatment time or dose.**

This study is classified as **Acceptable-Guideline**. It does satisfy the requirement for OPPTS Test Guideline 870.5375 for *in vivo* cytogenetic mutagenicity data.

870.5450 Dominant Lethal Assay

In a mouse dominant lethal assay (MRID 42637003), groups of 30 male Tif: MAGf (SPF) mice were treated orally by gavage with Atrazine technical (97.1% a.i., batch #SG8029BA10) at doses of 0, 500, 1000, 2000, or 2400 mg/kg body weight in a volume of 10 mL/kg. The vehicle was corn oil. Starting immediately after dosing, each male was mated with 2 untreated females per interval for days 1-4, days 4-8, and days 8-12. Each male was then mated with 2 untreated females per week for weeks three through eight.

Atrazine technical was tested at an adequate dose. There were signs of toxicity after dosing as evidenced by piloerection and decreased locomotor activity. The females were sacrificed on gestation day 13-15 and the uteri examined for the number of alive, early, and late dead embryos and resorptions. Cyclophosphamide served as the positive control.

There was no significant difference between the control group and treated groups with respect to post-implantation mortality of embryos. Under the conditions of this study atrazine technical did not induce dominant lethal mutations in male mice at doses as high as 2400 mg/kg.

This study is classified as **Acceptable- Guideline**. It does satisfy the requirement for OPPTS Test Guideline 870.5450 for rodent dominant lethal data.

870.5550 UDS Assay

In an unscheduled DNA synthesis assay (MRID 00161790/40722301) primary rat hepatocytes from adult male Tif:RAIf rats that were exposed to concentrations of atrazine of 0, 1.2, 6, 30, 150 : g/mL for five hours. **No concentration induced UDS, including the high dose of 150 : g/mL, where precipitation of the test article occurred.**

This study is classified as **Acceptable-Guideline**. It does satisfy the requirement for OPPTS Test Guideline 870.5550 for other genotoxic mutagenicity data.

870.5550 UDS Assay

In an unscheduled DNA synthesis assay (MRID 42547105), primary rat hepatocyte cultures were exposed to atrazine, (97.1% a.i.), in dimethyl sulfoxide at concentrations of 15, 46, 139, 417, 835, and 1670 µg/mL for 16-18 hours.

Atrazine was tested up to precipitating concentrations, 139 µg/mL. The positive controls did induce the appropriate response. **There was no evidence that unscheduled DNA synthesis, as determined by nuclear silver grain counts, was induced.**

This study is classified as **Acceptable-Guideline**. It does satisfy the requirement for OPPTS Test Guideline 870.5550 for other genotoxic mutagenicity data.

4.7 Neurotoxicity

Acute and subchronic neurotoxicity studies are not available for atrazine. Special studies submitted by the registrant (MRIDs 44152102 and 43934406) and published in the open literature (Cooper, *et al.* 2000. Atrazine disrupts the hypothalamic control of pituitary- ovarian function. Tox. Sci. 53: 297-307 [MRID 45166902]) provide evidence of atrazine-associated central nervous system (CNS) toxicity. The neurotoxicity seen in these studies was a CNS toxicity (specifically, neuroendocrine alterations at the hypothalamus).

4.8 Metabolism

Adequacy of data base for metabolism: The data base for metabolism is considered to be complete. No additional studies are required at this time.

870.7485 Metabolism - Rat

Several metabolism studies have been submitted to fulfill the 870.7485 guideline requirements. MRIDs 40431304; 40431305; 40431306; and 42165503 together satisfy the metabolism guidelines. MRID 44713802, by itself, satisfies the 870.7485 guideline requirements. These studies are summarized below.

In a metabolism study (MRID 40431304) [atrazine, chemical purity of unlabeled compound not given, radio-purity of labeled compound > 98%, all carbons on the triazine ring were labeled] was administered to 34 Sprague Dawley CD rats in four dose groups, 5/sex/dose, except controls which were 2 sex/dose. Three groups were dosed acutely at dose levels of 0, 1, and 100 mg/kg for a single dose given through oral gavage. A fourth dose group was dosed subchronically at 1.0 mg/kg/day for 15 days by oral gavage.

Distribution, accumulation
Distribution was dose-dependent and independent of sex. In the 100 mg/kg group, the highest distribution of radiolabel was in red blood cells followed by the heart, spleen, lung and liver. In the group given 1 mg/kg for 15 days distribution was highest in the red blood cell, followed by the liver, spleen, kidney and lung. Distribution appeared to follow first-order kinetics and the half-life in the tissues was 31.3 hours. This

indicates that atrazine does not bioaccumulate.

Excretion
Approximately 95% of the atrazine was excreted within 7 days of dosing. The urinary route accounted for about 75% of the excretion while feces accounted for 20%. The route of excretion did not seem to vary among sexes or with dose.

This metabolism study in the rat is classified **Acceptable-Nonguideline** and, *when considered in conjunction with studies: MRID Nos.: 404313-05; -06; and 421655-03,* satisfies the guideline requirement for a metabolism study (870.7485) in the rat. Only in conjunction with these four studies does MRID 404313-04 satisfy the metabolism guideline requirement.

In a metabolism study (MRID 40431305) Atrazine, 98.8% a.i., was labeled with ^{14}C on all carbons of the triazine moiety, radiopurity 97.9%, and was administered to 14 female Sprague-Dawley rats 2/dose. The animals were dosed daily through a stomach tube with dose levels of 0, 1, 3, 7, 10, 50 or 100 mg/kg/day. One animal from each group was sacrificed 3 hours and the other was sacrificed 72 hours following the last dose.

Distribution, accumulation
The location of ^{14}C label did not vary with dose but the amount distributed did vary with dose. Distribution was highest in the red blood cell, followed by the liver, ovary and kidney. When the dose increased the amount distributed in the tissues increased. The distribution appeared to follow first-order kinetics and the tissue half-life was 38.6 hours. This indicates that atrazine, with possible exception of the red blood cell, does not bioaccumulate.

This metabolism study in the rat is classified **Acceptable-Nonguideline** and, *when considered in conjunction with studies: MRID Nos.: 404313-04; -06; and 421655-03,* satisfies the guideline requirement for a metabolism study (870.7485) in the rat. Only in conjunction with these four studies does MRID 404313-04 satisfy the metabolism guideline requirement.

In a metabolism study (MRID 40431306) atrazine (purity of unlabeled compound not reported but radiolabeled purity >97%), ^{14}C-labeled on all carbon of the triazine moiety, was administered to 13 female Sprague-Dawley rats. The test article was given through the stomach tube in a single oral dose. Five animals were given 100 mg/kg and urine and fecal samples were collected 24, 48 and 72 hours later, after which the animals were sacrificed and 5 mL of blood and the liver were obtained. The remaining 8 animals were given between 16.18 and 19.64 mg/kg and urine was collected over a 24 hr period. The urine was analyzed for metabolites using anion/cation exchange chromatography or reverse phase HPLC followed by infrared and mass spectrometry.

Excretion
In the rats given 100 mg/kg, 100% of the administered radioactivity was accounted for within 3 days of dosing. Urine was found to contain 47.3% of the radioactivity and the feces 49.3%. The tissues contained 5.75% and 1.4% was found in the blood.

Metabolism

Metabolites detected from the 8 rats given 16.18-10.64 mg/kg indicated that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alky substituents of atrazine appears to be of minor metabolic importance.

This metabolism study in the rat is classified **Acceptable-Nonguideline,** and, *when considered in conjunction with studies: MRID Nos.: 404313-04; -05; and 421655-03*, satisfies the guideline requirement for a metabolism study (870.7485) in the rat.

In a metabolism study (MRID 42165503) fecal and urinary samples from rats exposed to 1 or 100 mg/kg radiolabeled atrazine in a separate metabolism study (MRID 40431304) were obtained and analyzed by thin layer chromatography to determine metabolism profiles. No sex differences in metabolic profiles were evident. The major fecal metabolite was DACT which accounted for 40% of the total fecal radioactivity. The major urinary metabolite was also DACT.

This metabolism study in the rat is classified **Acceptable-Nonguideline,** and, *when considered in conjunction with studies: MRID Nos.: 404313-04; -05; and -06*, satisfies the guideline requirement for a metabolism study (870.7485) in the rat

In a metabolism study (MRID 44713802), groups of male and female rats (3-12 per group depending on the experiment) were each given [U-^{14}C] Triazine G 30027 (batch no. GAN-XVIII-77-5; purity>95%) and non-radiolabeled Triazine (batch no. AMS 126/105; purity 99%) to provide a total single oral dose of 1 or 100 mg/kg. Urinary and fecal excretion were monitored up to 168 hours, tissue burdens were assessed up to 336 hours post dosing, and major metabolites quantified and, where possible, identified. Biliary samples were taken from four male rats up to 48 hours post dosing and metabolite analysis performed.

There were no treatment-related deaths. Recovery of administered radioactivity was 95.84% and 98.17%, respectively, for the administered high (168 hours postdosing) and low dose (48 hours postdosing). Based upon urinary and biliary excretion data, and tissue/carcass burden data, absorption was relatively rapid. The time to maximum blood concentration (t_{cmax}) was 2 hours and 24 hours for the low and high dose groups, respectively. Under the conditions of this study, absorption and elimination did not appear to be saturated. The major route of excretion was via the urine, accounting for 64.72% of the total administered low dose over a 48-hour period and 66.16% of the total administered high dose over a 168-hour period. Time-course studies revealed that within 48 hours urinary excretion was 100% and 94% complete for the low-dose and high-dose group, respectively. Fecal elimination accounted for 10.80% and 19.69% of the total dose for the low and high dose groups, respectively. Over a 48-hour period, biliary secretions accounted for 7.35% of the administered dose (68% of total amount excreted via the feces) in the low-dose group. Total fecal elimination of radioactivity accounted for 10.80% and 19.69% of the total administered dose, a portion of which was due to biliary secretion resulting from absorbed test material.

Tissue distribution appeared to be extensive and reached maximum concentrations (t_{cmax}) at 2 hours and 24 hours for the low-dose and high-dose groups, respectively. Time to half maximum tissue concentrations (½ t_{cmax}) for these groups were 48 hours and 168 hours, respectively. Although test article associated radioactivity was widely distributed, concentrations were greatest in red blood cells and highly perfused tissues and organs. With exception of red blood cells, whole blood, and skeletal muscle, tissue burden for any specific tissue or organ represented less than 1% of the administered dose by 14 days post dosing. Whole-body autoradiography confirmed the extensive distribution of the test material and/or its metabolites, and the rapid removal of radioactivity from the gastrointestinal tract. The pattern of tissue distribution did not appear to be significantly affected at the doses tested. Removal of radioactivity from tissues was a biphasic process characterized by a relatively rapid phase at 48 hours followed by a slower phase (48-336 hours). Depuration of radioactivity from red blood cells, however, exhibited a slower monophasic pattern over 336 hours.

Urinary, biliary and fecal metabolites were detected and revealed minimal dose-related qualitative differences in metabolite profile. Quantitative differences were not inconsistent with that expected for a 100-fold difference in dose. Twenty six urinary metabolites were detected although only two accounted for more than 5% of the administered dose. Chromatographic analysis using known reference standards allowed for identification of four of the urinary metabolites. Nine biliary metabolites were detected, although none of the biliary metabolites accounted for more than 1.6% of the total administered dose. Three biliary metabolites were also identified as urinary and fecal metabolites. Twelve metabolites were identified in the feces of high-dose rats and nine metabolites were identified in the feces of low-dose rats, none of which represented more than 2.42% of the total administered radioactivity.

This study adequately described the metabolism and disposition of Triazine in rats administered single oral doses of 1 or 100 mg/kg. Inventory of administered radioactivity was acceptable and metabolite profiles indicate that, at the doses tested, Triazine G 30027 is nearly completely metabolized. The conclusion of the study authors that the major metabolic pathway appears to be dealkylation to the major metabolite (G 28273) and that dechlorination to CGA 10582 is a minor pathway is consistent with the data presented in the study report.

This metabolism study in rats is **Acceptable/Guideline** and satisfies the requirements for a Metabolism and Pharmacokinetics Study (OPPTS 870.7485). This study (MRID 44713802) was properly designed and conducted, and provided data regarding the absorption, distribution, and elimination of the test material, and quantitation and identification of urinary, biliary, and fecal metabolites in rats given a single oral low dose (1 mg/kg) or high dose (100 mg/kg) of [U-^{14}C]Triazine G 30027.

870.7600 Dermal Absorption - Rat

In a dermal absorption study (MRID 43314302), male Charles River CD rats received either 0.01, 0.1 or 1 mg/cm^2 of ^{14}C-atrazine(two vials used: radiochemical purity: 98.7 and 98.9%, specific activity: 1.9 uCi/mg and 19.1 uCi/mg). Animals were exposed in three groups: animals in group one were exposed to a single dose of the 3 above listed concentrations for 0.5, 1, 2, 4, 10, or 24 hours and were sacrificed immediately following dosing; group two animals were exposed to a single dose of the above listed concentrations for 10 hours and were sacrificed 34, 58 or 82 hours following the start of exposure; and group three animals were exposed to a single dose of the above listed concentrations for 24 hours and then sacrificed 48, 72 or 96 hours following the start of exposure. The exposure sites were washed with liquid Dove and water at the end of the exposure period. The animals were kept in metabolism cages during exposure and after exposure until sacrifice.

The percent absorbed increased with time and decreased with dose. Regardless of the dose or exposure time, the majority (65 - 95%) of the radio labeled atrazine was recovered in the washes or was found associated with the skin at the site of exposure. The maximum percent absorbed was in the animals exposed to the low dose for 24 hours and sacrificed 96 hours following the start of exposure. Absorption was approximately 30% in this group. This indicates that much of what remains on the skin is subsequently absorbed.

The percent absorbed increased with time and decreased with dose. A significant portion of compound remaining on washed skin is subsequently absorbed given enough time.

This dermal absorption study in the rat is classified **Acceptable-Guideline**, and satisfies the guideline requirement for a dermal absorption study (870.7600) in the rat.

870.7600 Dermal Absorption - Human

In a dermal absorption study (MRID 44152114), 10 human volunteers were exposed to a single topical dose of [triazine ring-U-^{14}C] atrazine (94.3-96.3% a.i., 98.0-98.4% radiochemical purity) at 6.7 (4 volunteers) or 79 : g/cm^2 (6 volunteers) for 24 hours; equivalent to 0.1667 and 1.9751 mg of [14C] atrazine for the low and high doses, respectively.

Overall recoveries of radioactivity from the low- and high-dose groups were 101 and 92%, respectively. The majority (91.1-95.5%) of the dose remained unabsorbed and was detected in skin wash samples taken 24 hours after dosing. After 168 hours, only 5.6% of the dose was absorbed and excreted in the urine and feces of the low-dose group and only 1.2% in the high-dose group. The renal excretion half-life was 19.6-29 hours for the low-dose group and 25.9-31 hours for the high-dose group. In both dose groups, peak urinary elimination occurred at 24-48 hours and peak fecal elimination occurred at 48-72 hours.

Total chlorotriazine residues detected by TLC in a high-dose 0-24 hour composite urine sample accounted for 9.16% of the TRR and included deethyl atrazine (3.88% TRR) and didealkyl atrazine (5.28% TRR). No atrazine was detected. GC/MSD analysis of urine samples also did not detect atrazine or its chlorotriazine metabolites.

Enzyme immunoassays indicated that levels of atrazine mercapturate, an expected glutathione pathway metabolite was near the limit of detection/quantitation for the methods. Mercapturic acid conjugates of atrazine and its chlorotriazine metabolites were not detected by LC/MS/MS in urine samples.

Some similarities in HPLC profiles of urine from i.v. dosed monkeys (MRID 44152112) and dermally treated humans were observed.

This dermal absorption study in humans is **Acceptable-Nonguideline**. This study was not meant to satisfy an 870.7600 guideline.

4.9 Special/Other Studies

4.9.1 Assays of Direct Estrogenic Activity of Triazines

Study 1:

This study (MRID 43598617) combines three *in vivo* assays to investigate the estrogenic effects of atrazine, simazine, and diaminochlorotriazine [DACT, (a.i. > 96%)] on body weight, body weight gain, uterine weight, progesterone receptor binding capacity, and thymidine incorporation on female Sprague-Dawley rats.

For body weight and uterine weight measurements, ovariectomized rats were dosed by oral gavage daily for 3 days at 20, 100, and 300 mg/kg/day in single exposure experiment and in co-exposure experiments with 2 µg estradiol on day 2 and 3. Body weight gains were decreased for rats exposed to the vehicle control, 0.5% carboxymethylcellulose, (-3.5%) and to the positive control, 2 µg estradiol, (-6.9%). Larger decreases in weight gain were observed in rats exposed to triazines. At 20 mg/kg/day weight losses ranged from 6-9%. At 100 mg/kg/day, weight losses ranged from 8-13.5%. At 300 mg/kg/day, weight losses ranged from 11-17%.

Chemicals with estrogenic activity cause proliferation and thickening of the uterine wall. The uterine weights of rats exposed to triazines were similar to vehicle control uterine weights. In co-exposure experiments with estradiol, exposure to atrazine and DACT at 100 and 300 mg/kg/day resulted in a dose-dependant and statistically significant decrease in uterine weight relative to the positive control. In co-exposure experiments with estradiol, uteri of rats exposed to simazine at 100 and 300 mg/kg/day were significantly decreased relative to the positive control.

Thymidine incorporation was measured to assess the effect triazines on DNA synthesis in the uterus of exposed rats. Dose groups included: exposure to 300 mg/kg/day of triazine alone administered by oral gavage for 2 days; and exposure to 1,10, 20, 50, 100, and 300 mg/kg/day of triazine administered by oral gavage for 2 days plus an injection of 0.15 µg estradiol on day 2. Exposure to triazines alone at 300 mg/kg/day resulted in significant decrease relative to the vehicle control. Exposure to the positive control, 0.15 µg estradiol, resulted in approximately 2.5 fold increase ($p < 0.05$) in thymidine incorporation relative to the vehicle control. Exposure to 50, 100, and 300 mg/kg/day in co-exposure with 0.15 µg estradiol resulted in a significant reduction in thymidine incorporation relative to the positive control.

Levels of inducible progesterone receptor (PR) were indirectly measured by the PR binding capacity to a radioactive ligand. The PR was isolated from uteri of rats exposed by oral gavage to atrazine, DACT, and simazine alone at 300 mg/kg/day for 2 days and to atrazine, DACT, and simazine at 50 and 300 mg/kg/day in co-exposure with 1 µg/day estradiol for 2 days. The PR binding capacity in rat uteri exposed to 300 mg/kg/day triazine alone was statistically decreased relative to vehicle control. PR binding capacity decreased significantly relative to the positive control with exposure to atrazine, DACT, and simazine at 300 mg/kg/day in co-exposure experiments with 1 µg estradiol.

According to the effects on uterine weight, progesterone binding capacity, and thymidine incorporation at the concentrations tested in this study, atrazine, DACT, and simazine do not exhibit estrogenic activity.

This study is classified as **Acceptable-nonguideline** as a special study on *in vivo* and *in vitro* estrogenic effects.

Study 2:

In a special study (MRID 43598618) on *in vitro and in vivo* competitive binding of atrazine, diaminochlorotriazine (DACT, a mammalian metabolite of both atrazine and simazine) and simazine (>96 % a.i. for all three compounds) to the estrogen receptor (ER), several different experiments were performed utilizing extracted cytosolic ER from adult female Sprague-Dawley rats. Experiments observed the displacement of radiolabeled estradiol by atrazine, simazine, and DACT under both equilibrium (simultaneous exposure to triazine and radiolabeled estradiol at 4°C) and at disequilibrium conditions (pre-exposure to triazine at 25°C for 30 min. prior to exposure to radiolabeled estradiol). Results of equilibrium experiments indicated that atrazine, simazine, and DACT did not displace any radiolabeled estradiol whereas the positive control exhibited a dose-dependant displacement of the radiolabeled estradiol. Under disequilibrium conditions in a time-course experiment, uterine cytosolic extracts were first incubated with triazine at 100 µM for 30 min. prior to exposure to radiolabeled estradiol for 5-150 minutes. With time radiolabeled estradiol displaced triazine that had bound during the initial exposure. In a dose-response experiment under disequilibrium conditions, uterine cytosolic extracts were first incubated with triazine at concentrations 10^{-9} to 10^{-3} M or unlabeled estradiol at 10^{-11} to 10^{-7} M prior to exposure to radiolabeled estradiol. The concentration at which 50% inhibition of binding occurred (IC_{50}) for unlabeled estradiol was approximately 10^{-9} M whereas the IC_{50}'s for the triazine were 20 µM for atrazine and 100 µM for simazine and DACT.

An additional experiment under disequilibrium conditions was performed to mimic a Scatchard type analysis using constant molar excess of triazine (100X estradiol, 10,000X atrazine, or 10,000X simazine) relative to the radiolabeled estradiol (0.2 nM, 0.5 nM, 1.5 nM, and 5.0 nM). Results indicated the triazines competed with the radioligand better at lower concentrations of tracer. For example, there was 60% displacement of 0.5 nM radiolabeled estradiol when co-exposed with 5 µM atrazine whereas there was 11% displacement of 5 nM radiolabeled estradiol when co-exposed with 50 µM atrazine. In the Scatchard-type plot, dissociation constants were produced; 0.5 nM in the presence of 100-fold molar excess of estradiol and 1 nM in the presence of 10,000 fold molar excess of atrazine and simazine. The x-intercepts of the plot were approximately equal (210 fmol/mg) in the presence of estradiol and simazine indicating competitive binding. The x-intercept for the atrazine plot was slightly less (158 fmol/mg) indicating the potential for competitive binding but also some noncompetitive binding under the disequilibrium conditions of this study.

Experiments using uteri extracted with KCl to extract the total ER and without KCl to extract the ER not bound or only loosely bound to chromatin were performed using cytosol fractions incubated with 50 µM triazine or 500 nM estradiol under disequilibrium conditions followed by separation of the sample by sucrose density centrifugation. One hundred twenty five mL fractions were removed and counted for radioactivity. The authors generated plots representing the fraction number versus the radioactivity in the given fraction in order to observe which fraction exhibited the binding. In the extractions without KCl, the receptor binding peaks were detected in the 7-8S fraction for both estradiol and atrazine. Atrazine displaced an average of 10.7% of the radiolabeled estradiol whereas estradiol displaced all of the radiolabeled tracer. In the conditions favoring the extraction of total ER, activated and unactivated, binding was detected in the 4-5S fraction. Atrazine displaced an average of 29.6% of the radiolabeled estradiol whereas estradiol displaced all of the radiolabeled tracer.

Making the assumption that living rats contain the activated form of the ER, in order to test whether triazines competed more effectively against the transformed ER, ovariectomized rats were dosed with triazines prior to uterine dissection and competitive binding experiments. The binding of radiolabeled estradiol was reduced by an average of 33%, 39%, and 24% for atrazine, simazine, and DACT, respectively ($p < 0.05$ vs. vehicle controls) in the 300 mg/kg/day group. In the 50 mg/kg/day group, binding of radiolabeled estradiol was reduced (not statistically significant) by 18, 21, and 13% .

Overall the results indicate that atrazine, triazine, and DACT do exhibit some competitive binding with estradiol but only under conditions which favor triazine binding.

This special study on *in vitro and in vivo* competitive binding of atrazine, DACT and simazine binding to the estrogen receptor in the rat is **Acceptable-nonguideline.**

Study 3:

Another study (MRID 43598619) combines the following *in vitro* assays to investigate the estrogenic and antiestrogenic activity of atrazine and simazine: binding to hepatocyte Ah receptor; proliferation of MCF-7 (human breast cancer cell line) cells; gel electrophoresis mobility shift assay to measure levels of progesterone receptor; and MCF-7 transfection using a luciferase reporter gene.

Competitive binding to the aryl hydrocarbon (Ah) receptor was measured using 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as the competitor. This assay is performed because some dioxins, which are strong Ah receptor agonists, have also been shown to exhibit antiestrogenic activity. Cytosol from male rat hepatocytes containing Ah receptor was isolated and incubated with the positive control, radiolabeled TCDD, plus either atrazine or simazine. With addition of hydroxylapatite to this mixture, high molecular weight compounds, such as test compound bound to the Ah receptor, will precipitate out of solution. Subsequent centrifugation of this solution will result in the Ah receptor, and anything bound to it, being spun out into a pellet. By counting the radioactivity in the pellet, it is possible to measure the amount of radiolabeled TCDD displaced indicating competitive inhibition with the triazine. Competitive binding was measured as the decrease in radioactivity versus the positive control. At concentrations up to 10,000 nM, atrazine and simazine did not displace TCDD from the Ah receptor.

A cell proliferation assay using the MCF-7 cell line was performed using the following doses: 10 µM, 1.0 µM, 0.1 µM, and 0.01 µM for atrazine and simazine and 1 nM for estradiol. Experiments using individual chemicals plus triazine were performed. Results indicated that the positive control increased the density of MCF-7 cells two fold relative to the negative control after 11 days . Exposure to atrazine and simazine alone neither increased nor decreased cell number at day 11 relative to the negative control. Results of co-exposure experiments indicate that cell number did not change relative to the positive control.

Estradiol has been shown to increase the level of progesterone receptor in mammalian cells. This assay measures the effect of estradiol and/or triazine on the binding capacity of a high affinity ligand (R5020) to the progesterone receptor which is an indirect measure of progesterone level. The gel electrophoresis mobility shift assay utilized indirectly measures progesterone receptor (PR) levels in MCF-7 cells following exposure to atrazine, simazine, or estradiol for 3 days. Polyacrylamide gel electrophoresis can separate molecules, such as DNA, or complexes of molecules, such as protein-DNA complexes, based on size.

The DNA used in this study is a radioactive oligonucleotide of a specific sequence which binds to the PR. This sequence is called a progesterone response element (PRE). PR isolated from MCF-7 cells is first incubated with R5020. PR-R5020 complex was then incubated with radiolabeled PRE. The R5020-PR-PRE complex was then run on a polyacrylamide gel. The unbound PRE was separated from the bound complex based on differences in mobility through the gel. Doses used were 1 µM for atrazine and simazine and 10 nM for estradiol. Exposure to estradiol resulted in a 3.5-fold increase in reactivity of the R5020-PR-PRE complex relative to the negative control. The radioactivity in the R5020-PR-PRE band following exposure to atrazine and simazine was similar to negative control. The radioactivity in the band representing the R5020-PR-PRE complex was measured to get an indirect measure of PR levels in MCF-7 cells. Results indirectly indicate that the levels of PR did not increase or decrease with exposure to atrazine or simazine.

Transfection experiments were performed where the human estrogen receptor along with the luciferase expression system were inserted into MCF-7 . The MCF-7 cell assay measured luciferase activity (*i.e.*, light production) and is quantitative in nature (*i.e.*, concentration of estrogenic chemical is directly related to light production). Doses used in the luciferase experiments were 10^{-13} M to 10^{-8} M estradiol and 10^{-9} M to 10^{-5} M atrazine and simazine. Atrazine and simazine did not induce luciferase activity above background at concentrations as high as 10^{-5} M (concentrations above 10^{-5} M are toxic to MCF-7 cells). Exposure to the positive control, estradiol, resulted in significant luciferase activity beginning at 10^{-12} M. Light production increased exponentially up to 10^{-8} M where light production plateaued.

Neither atrazine nor simazine displayed estrogenic activity or interacted with the Ah receptor in the set of experiments described in this paper.

This study is classified as **Acceptable-nonguideline** as a special study on *in vitro* estrogenic effects.

Study 4:

Another study (MRID 43934403), combines three *in vivo* and four *in vitro* assays to investigate the estrogenic and antiestrogenic activity of atrazine and simazine. *In vivo* experiments, 4 or 5 female Sprague-Dawley rats/dose were dosed orally or by intraperitoneal injection. In order to test for estrogenic activity, rats were exposed to atrazine (> 97% a.i.) or simazine (> 97% a.i.) at 50, 150, and 300 mg/kg/day or estradiol (positive control) at 10 µg/kg/day. In order to test for antiestrogenic activity, co-exposure experiments using atrazine or simazine plus estradiol were performed using the same concentrations. Following a three day exposure, the following *in vivo* endpoints were measured: uterine weight, progesterone receptor levels and uterine peroxidase.

Exposure to estradiol results in statistically significant increased uterine weights, increased levels of the progesterone receptor, and increased activity of uterine peroxidase. Exposure to atrazine and simazine individually resulted in negative effects indicating antiestrogenic activity are shown in slightly reduced uterine weights, statistically significantly reduced levels of the progesterone receptor, and statistically significantly reduced activity of uterine peroxidase.

The following *in vitro* assays were performed: proliferation of MCF-7 (human breast cancer cell line) cells; gel electrophoresis mobility shift assay to measure levels of progesterone receptor; MCF-7 transfection using the luciferase reporter gene; and yeast transfection using a selective media expression system.

Doses for the cell proliferation assay when tested individually and in combination were 10 µM, 1.0 µM, 0.1 µM, and 0.01 µM for atrazine and simazine and 1 nM for estradiol. Results indicated that the positive control significantly increased the density of MCF-7 cells three fold relative to the negative control after 11 days . Exposure to atrazine and simazine alone neither increased nor decreased cell number at day 11 relative to the negative control. Results of co-exposure experiments indicate that cell number did not change relative to the positive control .

The gel electrophoresis mobility shift assay used in this study indirectly measures progesterone receptor (PR) levels in MCF-7 cells following exposure for 3 days. The oligonucleotide used contains progesterone response element (PRE) which binds to the PR. PR from MCF-7 cells was isolated and incubated with radiolabeled PRE. The PR-PRE complex was then run on a polyacrylamide gel to separate bound and unbound PRE based on differences in mobility through the gel. The radioactivity in the band representing the PR-DNA complex was measured as an indirect measure of PR levels. Doses used were 1 µM for atrazine and simazine and 10 nM for estradiol. Results indicated that exposure to estradiol resulted in a 3.5-fold increase in reactivity of the DNA-PR complex relative to the negative control. The radioactivity in the DNA-PR band following exposure to atrazine and simazine was similar to negative control. These results indicate indirectly that the levels of PR did not increase or decrease with exposure to atrazine or simazine.

Two transfection experiments were performed where the human estrogen receptor was inserted into the genome of MCF-7 and yeast cells. The MCF-7 cell assay measured luciferase activity as a surrogate measure of estrogen receptor activity. Doses used were 10^{-13}M to 10^{-8} M estradiol and 10^{-9} M to 10^{-5} M atrazine and simazine. Atrazine and simazine did not induce luciferase activity above background at concentrations as high as 10^{-5} M (concentrations above 10^{-5} M are toxic to MCF-7 cells). Exposure to the positive control, estradiol, resulted in significant luciferase activity beginning at 10^{-12} M and increased exponentially up to 10^{-8} M where light production plateaued. Atrazine and simazine did not exhibit in estrogenic or antiestrogenic activity and did not interact directly with the estrogen receptor.
In the yeast transfection assay, the human estrogen receptor linked to a necessary amino acid was inserted into the yeast. Growth on selective media is a qualitative measure of activity of estrogen receptor. Yeast were dosed with 1 nM estradiol or 10 µM atrazine or simazine. Yeast proliferated when exposed to estradiol but did not proliferate when exposed to atrazine or simazine. These results indicate that atrazine and simazine did not exhibit estrogenic activity and did not directly interact with the estrogen receptor.

In conclusion, the results of these experiments indicate that *in vivo* atrazine and simazine ~~exhibits~~ some antiestrogenic activity, but no estrogenic activity. Based on the *in vitro* results, this antiestrogenic activity is not the result of *direct* interaction with the estrogen receptor.

This study is classified as **Acceptable-nonguideline** as a special study on *in vivo* and *in vitro* estrogenic effects.

4.9.2 Estrous Cycle Alterations and LH Surge Attenuation

1. Pilot study

In a study (MRID 43934404) to determine the ~~determine the~~ validity of a proposed protocol for testing the effect of atrazine exposure on the proestrus afternoon luteinizing hormone (LH) surge, estradiol (E_2) was administered to 70 ovariectomized female Sprague Dawley rats through a surgically implanted silastic capsule. Serum LH, E_2, and prolactin levels were measured 3 days later to determine if the LH and prolactin surges could be measured and to evaluate success of the estradiol implantation procedure.

The ovariectomy followed by estradiol implantation procedure appeared to be effective in inducing LH and prolactin surges, as both these surges were clearly evident in rats undergoing this procedure.

This special study in the rat is **Acceptable-nonguideline**. This study does not satisfy any guideline requirements and was not submitted with the intention of satisfying a guideline requirement.

2. 28 day study

In a study to evaluate the effect of atrazine exposure on the proestrus afternoon luteinizing hormone (LH) surge (MRID 43934406) atrazine, 97.1% a.i., was administered to 450 female Sprague Dawley rats in the diet. Dose levels were 0 (vehicle control), 2.5, 5, 40 and 200 mg/kg/day for 28 days.

Mortality, clinical signs, gross pathology and pituitary weights were not affected in this study. Food consumption and body weights are decreased at the 40 and 200 mg/kg/day doses (body weight decreased 13% and 47% at 40 and 200 mg/kg/day respectively). The number of animals with diestrus blocks were increased at 40 and 200 mg/kg/day. The number of animals with estrus blocks were increased at 200 mg/kg/day. The prolactin surge was attenuated at 200 mg/kg/day. The LH surge was attenuated at 40 and 200 mg/kg/day [non-repeat bleed rats].

The LOAEL is 40 mg/kg/day, based on decreases in food consumption, body weight, body weight gain, estrous cycle alterations and LH surge attenuation. The NOAEL is 5 mg/kg/day.

This special study in the rat is **Acceptable-nonguideline**. This study does not satisfy any guideline requirements and was not submitted with the intention of satisfying a guideline requirement.

3. Six month study

In a study to evaluation the effect of long-term atrazine exposure on the proestrus afternoon luteinizing hormone (LH) surge (MRID 44152102) atrazine, 97.1% a.i., was administered to 360 female Sprague Dawley rats in the diet. Dose levels were 0 (negative control), 25, 50, and 400 ppm (0, 1.80, 3.65, 29.44 mg/kg/day) for 26 weeks (approximately six months).

Body weight, body weight gain and food consumption were significantly (p#0.05) decreased in ***animals at the highest dose tested*** [HDT] compared to controls (body weight decreased 8.5% at the end of the study and food consumption decreased 3.75% for the entire study). The percentage of days in estrus were significantly increased (p#0.01) during the 21-22 and 25-26 week time periods at the HDT. Percent days in estrus were also increased during the 21-22 and 25-26 week time periods at the mid dose [MDT], but the increase was only significant (p#0.05) for the 21-22 week time period. The proestrus afternoon LH surge was severely attenuated at the HDT (LH levels at most sampling time points were actually decreased compared to baseline) and less so at the MDT (maximum increase over baseline was 157% compared to maximum increase over baseline in controls of 273%). Pituitary weight were increase at the HDT (absolute weight increased 22% and weight relative to body weight was increased 28%). Pituitary weights at the other two doses were not affected. There was a slight increase at the HDT of animals displaying enlarged pituitaries (0% in controls compared to 3.4% at 29.44 mg/kg/day) and thickened mammary glands (0% in controls compared to 6.7% at 29.44 mg/kg/day). There were no other gross necropsy findings in the HDT that could be attributed to compound exposure and there were no compound-related gross pathology findings at the MDT or lowest dose tested [LDT]. Selected tissues were saved for histopathology but those results have yet to be reported.

There were no compound related effects in mortality or clinical signs. The proestrus afternoon prolactin surge was not affected by compound exposure at any dose. The LDT had no effects on the estrous cycle, LH or prolactin surges.

The LOAEL is 3.65 mg/kg/day, based on estrous cycle alterations and LH surge attenuation. The NOAEL is 1.8 mg/kg/day.

This special study in the rat is **Acceptable-nonguideline**. this study does not satisfy any guideline requirements and was not submitted with the intention of satisfying a guideline requirement.

4.9.3 Other Two-Year Bioassays

Three other two-year bioassays have been submitted to the Agency. These are: 1. a serial sacrifice study using Sprague-Dawley female rats (Study 1, below); 2. a study using Sprague-Dawley female rats with two dose groups and a control (Study 4, below); 3. a serial sacrifice study using F-344 female rats (Study 5, below).

Certain data collected in the two serial sacrifice studies were submitted separately and have their own MRID numbers. Hormone measurements and estrous cycle data from both SD and F-344 bioassays were submitted under the MRID number 42743902. Histomorphology data from these two bioassays was submitted under the MRID number 43598622. The table below displays the MRID numbers for each submission.

SD Serial Sacrifice Bioassay		F-344 Serial Sacrifice Bioassay	
Measurements	**MRID #**	**Measurements**	**MRID #**
Body wt., food cons., clinical observations, gross pathology	42085001	Body wt., food cons., clinical observations, gross pathology	42146101
Hormone measurements and Estrous cycle measurements	42743902	Hormone measurements and Estrous cycle measurements	42743902
Histomorphology	43598622	Histomorphology	43598622

Study 1 (SD serial sacrifice bioassay):

In a 2-year bioassay (MRID 42085001) seventy SD female rats (no males were used) were exposed, through the diet, to doses of Atrazine (97%) at 0, 70 and 400 ppm (0, 4.23 and 26.23 mg/kg/day) for 2 years. Ten females per dose were sacrificed at 1, 3, 9, 12, 15, 18 and 24 months. Vaginal smears were taken for two weeks prior to each sacrifice date and serum was collected for measurements of estradiol, progesterone, prolactin and corticosterone, on the day of sacrifice.

Mortality was increased in a dose-dependent manner. There were five unscheduled deaths in the control group, 6 in the 70 ppm group and 8 at 400 ppm. Using the Gehan-Breslow test there was a statistically significant (SS) negative trend for survival (survival decreased as the dose increased). Another statistical test - the Cox-Tarone test - did not indicate a significant trend in either direction. Body weights at several timepoints in the 400 ppm group were SS reduced compared to controls. Food consumption was also SS reduced at several timepoints in the 400 ppm group compared to controls. Body weights and food consumption did not appear to be altered in the 70 ppm group compared to controls.

Atrazine treatment at 26.2 mg/kg/day increased mortality, decreased body weights and decreased food consumption.

Study 2 (hormone measurement and estrous cycle evaluations in SD rats):

In a special study (MRID 42743902) female SD rats from the two year bioassay with serial sacrifices described above under "Study 1" (MRID 42085001) had their estrous cycles measured and serum levels of estradiol, progesterone, prolactin and corticosterone measured prior to or at each serial sacrifice. The results of these estrous cycle evaluation and serum hormone measurements are summarized below.

Estrous cycle evaluations (vaginal smears)

The percent days spent in estrus in control animals increases as the animals age at the expense of days spent in diestrus. The percent days of the cycle spent in estrus was dramatically increased in dosed animals compared to controls at nine, 12 and 18 months. Females in the 4.23 and 26.23 mg/kg/day groups at nine months spent an average of 34.3 and 44.8% of their days in estrus, respectively ($p<0.05$ at 70 and $p<0.01$ at 400 ppm). This is compared to 24% spent in estrus at the controls. The dose-related trend, as determined using a Terpstra-Jonckbeere Trend Test, showed a significant increase in % days spent in estrus ($p<0.01$). At 12 months there was also a dose-related trend ($p<0.05$) but the increases in dosed groups compared to controls as determined by ANOVA were not significant at either dose.

Atrazine-treated animals showed an early onset of increased days in the estrus phase of the estrous cycle. This effect occurred at both doses in this study.

Hormone measurements

Serum progesterone and corticosterone levels did not show any significant dose-related alterations compared to controls. Serum estradiol levels in the control rats in this study showed a positive trend (levels increased as time increased) over months one through nine. Exposure to atrazine did not alter this trend. The 4.23 and 26.23 mg/kg/day dose groups also showed a significantly positive trend from months one through nine. These trends are expected as constant estrus would be expected to begin to set in as these animals approach nine months of age. Examination of the pairwise comparisons at three months indicates that treated animals had an early onset of increased serum estradiol levels compared to controls. At three months control estradiol levels were 3.5 ng/mL, 70 ppm levels were 11.2, and 400 ppm levels were 16.2 ng/mL. The increase at 70 ppm was significant at $p<0.05$, the increase at 400 ppm was significant at $p<0.01$ and the trend, determined using a Terpstra-Jonckbeere Trend Test, was positive at $p<0.05$. At nine months control and 4.23 mg/kg/day group estradiol levels were similar, but the 26.23 mg/kg/day group compared to controls was increased 44%. At nine months estradiol levels were elevated compared to control, but not significantly so.

An early onset of increased serum estradiol levels is seen in both doses of atrazine-treated females.

Study 3 (histomorphology in SD):

In a special study (MRID 43598622) female SD rats from the two year bioassay with serial sacrifices described above under "Study 1" (MRID 42085001) had certain tissues, including their ovarian and mammary gland tissues, examined histopathologically. An evaluation was made of parameters in the ovarian tissues which indicated the animals current and recent estrous cycle states. The mammary gland was examined by histopathology for indications of exposure to steroid hormones such as estrogens, progesterone and prolactin. The examination of tissues, through histology, for morphologic alterations which provide information about certain aspects of an animals' physiologic state (such as the animal's estrous cycling ability or their exposure to hormones) is termed histomorphology. The results of the ovarian and mammary gland histomorphology from evaluations from this study are summarized below.

Ovarian Gland Histomorphology:

Selected tissues (including both ovaries and mammary glands) were removed and preserved at the serial sacrifice intervals described above under study 1, MRID 42085001. Several ovarian parameters were evaluated including the number and type of corpora lutea (CL) and the number of antral follicles. The presence of many "old" CL indicates that an animal went through normal estrous cycles in the past. A complete absence of CL indicates that an animal is not cycling and has not gone through an estrous cycle in some time. Antral follicles are mature follicles that are close to ovulation. An increased number of these means that they are accumulating and implies that the signal for ovulation (an LH surge) may not be available or of sufficient magnitude to stimulate ovulation.

There were no differences in number of old CL or antral follicles between controls and dose groups at the one month time point. By three months the number of animals which did not have old CL present increased in a slight, but dose-dependant manner, from zero in controls to one at 4.23 mg/kg/day and two at 26.23 mg/kg/day. Of the animals that had CL, there was an increased incidence in the dose groups of animals with reduced numbers of CL. Twenty percent of the controls were found to have reduced numbers of CL compared to 33% at 4.23 and 37.5% at 26.23 mg/kg/day. The mean grade per animal for antral follicles (*i.e.*, the sum total of all grades for all animals in the group divided by ten) was 2.1 and 2.2 in the 4.23 and

26.23 mg/kg/day groups, respectively, compared to 1.2 for controls. A higher mean antral grade indicates anovulation. These differences between control and treated groups became more extreme at the nine month timepoint. Seven of ten and ten of ten animals at nine months in the 4.23 and 26.23 mg/kg/day dose groups, respectively, had no CL at all present in their ovaries. This is compared to six of ten animals in the controls which did not have CL present. The mean antral follicle grade was 3.1 and 3.8 at the 4.23 and 26.23 mg/kg/day dose groups, respectively. This is compared to 2.6 in the controls. Data from the 12 month timepoint indicated that control animals had equalized with the treated animals in terms of parameters representing anovulation. Ten of ten controls and ten of ten high dose animals displayed no CL at 12 months while eight of ten low dose animals displayed no CL. The mean antral follicle scores were 3.3, 2.9 and 3.1 for the control, low dose, and high dose respectively.

Compared to controls there was an early onset in dosed groups, at three and nine months, of parameters such as absence of old CL, absence of any CL, CL reduced in number, and increased numbers of antral follicles. These parameters indicate lack of ovulation and the appearance of these parameters in greater numbers in dose groups than in controls, at three and nine months, provides evidence that atrazine exposure is associated with and early onset of anovulation in the this assay.

<u>Mammary Gland Histomorphology</u>:

Mammary gland histomorphology for several parameters is discussed below. Index weighted scores are used to describe the results of the histomorphic evaluations. An index weighted score assigns a numerical value to the severity of the finding assigned by the pathologist. The higher the index weighted score, the more severe was the finding in that group. These results show an early onset of increased index weighted scores for several histomorphic parameters which indicate exposure of the mammary tissue to estrogens, progesterone and prolactin.

Acinar Development - An early onset of increased exposure to estrogen is indicated by acinar development histomorphology index scores indicate and early onset of this finding in atrazine-exposed female SD rats. The index scores at the one-month time point are slightly higher in the dose groups than in the control, but a dose-response relationship was not seen. At the three- month time point the index scores are again increased over control. The increase is at this time point is dose-related though with the high dose being more severe than the low dose. At both nine and 12 months the index score again indicate more severe acinar development in the dose groups compared to the controls with the increase in severity being especially obvious at the high dose.

Acinar/Lobular Development - An early onset of this parameter is evident. The one- and three-month timepoints have index scores in the dose groups that are similar to the control index scores. The dose group index scores are clearly increased compared to controls at nine and 12 month timepoints though. This indicates that the atrazine-treated animals were exposed to elevated levels of prolactin at an earlier time point than the control animals.

Secretory Activity - The index scores for secretory activity also demonstrate an early onset of increased prolactin exposure in the dose groups compared to the controls. Index scores at one and three months in the dose groups were similar to control values, while index scores in both dose groups were clearly elevated compared to controls at the nine- and 12-month timepoints.

Dilated Ducts with Secretion - Index scores at one and three months in the dose groups were similar to control values. The index scores for the 4.23 mg/kg/day group compared to controls were only slightly elevated. The index scores for the 26.23 mg/kg/day group were greatly elevated compared to controls

Galactocele Incidence and Severity - No galactoceles were observed in any group at the one- and three-month timepoints. At nine and 12 months galactoceles in the dose groups were increased in both number and severity. By 15 months galactocele incidence and severity were similar between control and dose groups.

An early onset of several mammary gland histomorphology parameters in atrazine exposed SD rats of both doses in this study indicates an early onset of exposure to increased levels of steroid hormones - particularly estrogens and prolactin.

Study 4 (two dose two-year bioassay):

Atrazine technical was given in the diet to groups of 60 (female only) Sprague-Dawley rats for 24 months (MRID 42204401) at concentrations of 0, 70, or 400 ppm (approximating 0, 3.79, 23.01 mg/kg/day respectively). Water and food were available *ad libitum*. Test doses were selected following a 2-year study using 0, 10, 70, 500 or 1000 ppm of atrazine in the diet (MRID 00158930).

A slight reduction (a negative trend) in survival found amongst the dosed groups was considered to be equivocal because the data were statistically significant ($p<0.05$) by the Gehan Breslow test but not by the Cox-Tarone test. No treatment-related increases in clinical signs were noted in the study. Body weight gains were statistically significantly reduced relative to controls (approx. 12 to 13%) only at 400 ppm during weeks 0-76. Food consumption was only minimally reduced at the highest dose. Slight alteration in red blood cell shapes and incidence of nucleated RBCs were transient in occurrence. Spleen weights were slightly increased but the increase was not statistically significant. Other organ weight and organ:body weight values from the 400 ppm group were not significantly different from controls. nonneoplastic lesion findings were comparable in controls and treatment groups. Palpation times for tumors confirmed histologically indicated an early onset of mammary tumors. No statistically-significant increase in mammary tumors was observed.

The NOAEL for systemic toxicity is 70 ppm (calculated by the reviewer to be approximately 3.79 mg/kg/day) based on body weight gains of this group being 12-13% less than controls as well as statistically significant decreases in body weights in the 0-76 week period. Also, a reduction in survival considered to be equivocal is reported at 400 ppm. An MTD and effect level was determined in a previous chronic feeding study (MRID 00158930).

The study is classified Acceptable-nonguideline. This study alone does not fulfill Guideline requirements 870.4300 for carcinogenicity evaluation because only 2 dose levels were tested. Other studies have completed this Guideline requirement.

Study 5 (Intact vs OVX females and long-term estrous cycle evaluations):

In a carcinogenicity study intended to provide information about the mode of action for oncogenicity in atrazine exposed Sprague-Dawley rats,(MRID 44544701), atrazine, [97.1% a.i.] was administered to 800 female Sprague-Dawley rats. The rats were divided into 2 groups of 400 each. One group was ovariectomized (OVX) while the other was left intact. Atrazine was mixed with the diet at dose levels of 0 (control) 25, 50, 70 and 400 ppm (0, 1.5, 3.1, 4.2, 24.4 mg/kg/day for intact animals and 0, 1.2, 2.5, 3.5, and 20.9 mg/kg/day for ovariectomized animals) for 2 years.

Hematology, clinical chemistry, and urinalysis were not assessed in this study. Food consumption in the dose groups compared to the controls was not altered by compound exposure. The trend for survival was statistically-significantly decreased in the dosed groups compared to the controls. Survival was as follows: 43.3% in controls; 31.7% - 25 ppm; 28.8% - 50 ppm; 31.6% - 70 ppm; 21.7% 400 ppm. Body weight was statistically-significantly reduced in the first half of the study in the 400 ppm group (other groups were not significantly altered), but by the end of the study body weights were similar to control values. Organ weights for pituitary, uterus and the ovaries were taken in this study. No organ weights in either the intact or ovariectomized group were altered by compound exposure.

The only gross necropsy finding which was altered by compound exposure was the occurrence of mammary masses in the intact dosed animals. Dosed animals showed a higher incidence of mammary masses (many of which were confirmed by histopathology to be tumors).

There were no non-neoplastic findings at histology that were increased in dosed OVX animals compared to controls. OVX animals in all groups displayed very high incidences of juvenile uterus and castration cells in the pituitary which would be expected in an OVX animal. The only finding in dosed intact animals which was increased in incidence over controls were ovarian cysts which were slightly increased at 70 and 400 ppm compared to controls. There were though, many findings which were prevalent in the intact animals, yet were not seen (or were seen at lower levels) in the OVX animals. Mammary gland galactoceles were seen in anywhere from 65 to 78% of the intact animals, depending on the dose group, but the highest percentage of galactoceles in the OVX group was 24% in the 50 ppm group. Mammary gland secretory activity was seen in from 34 to 46% of the intact animals but was not seen in any OVX animals. Uterine dilation was seen in from 8-18% of the intact animals and uterine cystic endometrial hyperplasia was seen in about 50% of the intact animals. No OVX animals displayed uterine dilation and only about 20% displayed cystic endometrial hyperplasia. Intact animals also had increased incidence of pituitary findings compared to OVX animals. From 65 to 73% of the intact animals were found to have sinusoid ectasia/angiectasis but the range in OVX animals was 31 to 45%. The differences in mammary gland, uterine and pituitary findings between OVX and intact animals may provide information about the mode of action of atrazine's carcinogenicity.

Neoplastic histopathology findings were mostly limited to the pituitary and the mammary gland. Neither OVX nor intact animals showed an increase in pituitary tumors compared to their respective controls, but intact animals did show a 20-30% greater incidence of pituitary adenomas compared to OVX animals.

There were few mammary tumors in the interim sacrifice animals, which is not surprising given that these animals were sacrificed after only one-year. Excluding the interim sacrifice and looking only at those animals which were sacrificed at 24 months and those which died prematurely, there was an increase in mammary tumor incidence at all intact dose groups compared to controls. In ascending order of dose the percentage of animals with any type of mammary tumors was: 38.3% in controls; 53.3%; 71.2%; 56.6% and 68.3% at 400 ppm. Looking at carcinomas alone values are: 18.3%; 36.7%; 33.9%; 20%; and 41.7%. Fibroadenomas alone were: 26.6%; 40%; 52.5%; 45%; and 40%.

A decrease in the time-to-tumor is also evident from exposure to atrazine. In the control group 50% of the tumors occurred in the last 6 months of the study. The percentage of tumors which appeared in the last 6 months of the study in the dose groups were: 35.8%; 37.5% 36.5% and 33.4%. The number of tumors which occurred in the first year of the study was slightly increased at 25, 50 and 760 ppm and greatly increased at 400 ppm: 9% in controls; 10.7%; 10%; 12% and 17.9% in the 400 ppm group.

An increased incidence of mammary tumor in intact animals was observed at doses of 50 ppm (3.1 mg/kg/day) and above.

The purpose of this study was to examine mammary tumor carcinogenesis in female Sprague-Dawley rats. Thus, a large part of this review focuses on carcinogenicity.

One of the more striking aspects of the study was the complete lack of mammary tumors in OVX animals. Not a single mammary tumor of any sort was seen in any OVX animal. The lack of mammary tumors in OVX animals provides evidence that the mode of action of atrazine is neither a direct genotoxic nor estrogenic effect on the mammary gland. Rather, an indirect hormonally-mediated effect involving the ovary is implied.

At the 50 ppm and above doses, there was a treatment related increase in mammary tumor incidence when compared to controls. Dosing was considered adequate based on decreases in body weight. Additionally, there was a decreased time-to-tumor at doses of 25 ppm and above.

This carcinogenicity study in the rat is **Acceptable-nonguideline**, and *does not* satisfy the guideline requirement for a carcinogenicity study (870.4300) in the rat nor was it submitted with the intention of satisfying a guideline.

Study 6 (F-344 serial sacrifice bioassay):

In MRID 42146101 , seventy F-344 rats (females only) per dose were exposed ad libitum to diet that had been mixed with atrazine (97.1%) to the appropriate doses of 0 (negative control), 10, 70, 200 and 400 ppm (0, 0.68, 4.82, 14.05, 34.33 mg/kg/day). Ten animals per dose group were sacrificed after approximately one, three, nine, 12, 15, and 18 months exposure to the test article.

There was not an increase in mortality due to compound exposure, and there was no increased incidence of clinical signs in dosed animals compared to controls. The doses tested appeared to be sufficiently high because there was a decreased absolute body weight and body weight gain in the 400 ppm group compared to the controls. Group mean absolute body weight in the 400 ppm group compared to controls was also significantly decreased compared to controls at several time points though the final mean body weight was not significantly decreased compared to controls. The final group mean body weight for the 400 ppm group was 6.6% less than the mean control value. During the course of the study the 400 ppm animals gained an average of 116.7 grams compared to the weight gain in the control group of 133.3 grams (14% less than controls). This difference in body weight gain was statistically-significant at a p value of 0.05.

There was not an increase in mammary tumors or any other type of tumor at any dose group in either sex.

Studies 7 and 8, described below, are studies which performed hormone measurements, estrous cycle evaluations, and histomorphological evaluations on ovarian and mammary tissues in animals from this study.

Study 7 (hormone measurement and estrous cycle evaluations in F-344):

The hormone measurements data and estrous cycle evaluation results (vaginal smears) from the animals in MRID 42146101 are shown in MRID 42743902. HED believes that the estrous cycle evaluation data reported in MRID 42743902 are unreliable. The histomorphology data from MRID 43598622 are instead used to determine stage of the estrous cycle.

The results of the hormone measurements did not reveal any consistently statistically-significant alterations in serum hormone levels compared to controls for any of the hormones tested. There were occasional significant alterations such as significantly decreased ($p < 0.05$) progesterone levels in the 4.82 mg/kg/day dose and corticosterone levels in the 34.33 mg/kg/day group at the 12-month time point compared to controls; significant negative trends in estradiol levels at the 12-month time point, progesterone levels at the 12- and 18-month timepoints, and corticosterone levels at the 12- and 15-month timepoints, and prolactin levels displayed a significant positive trend at the three-month time point only. Careful consideration of these alterations indicated that they did not appear to be related to atrazine exposure.

There were alterations in serum hormone levels that were seen in control as well as treated rats. These were: decreases in estradiol levels in the later half of the study; significantly increasing progesterone levels from one to 18 months. Decreased estradiol and increased progesterone are expected in rats undergoing a reproductive aging process involving pseudopregnancy. Exposure to atrazine did not alter the age-related changes in estradiol or progesterone levels.

An increase in prolactin levels might be expected in an aging rat undergoing pseudopregnancy. However, consistent increases in serum prolactin levels were not seen.

Study 8 (histomorphology in F-344):

The histomorphologic evaluation of tissue from animals in MRID 42146101 (study 6, described above) is presented as MRID 43598622.

Ovarian Histomorpology: The great majority of F-344 rats in all groups, both control and dose groups, maintained corpora lutea (CL) throughout most of the study. Only at the final, 24-month, time point were there dramatic decreases in CL numbers. The atrazine-treated animals at this time point did not show decreases in CL numbers any more severe than the control animals. The reduction in CL numbers at this late time point appears to be a consequence of a natural progression of the animals from persistent diestrus into acyclicity. All animals in all dose groups maintained moderate numbers of secondary, antral and atretic follicles throughout the study - including the 24-month time point.

The ovarian histomorpohology seen in this study is what would be expected in a normally aging female F-344 rat undergoing a normal aging process towards a state of repetitive pseudopregnancy. Repetitive pseudopregnancy is associated with vaginal smears that are primarily leukocytic and is, thus, sometimes referred to as "persistent diestrous".

The ovarian histomorphology data indicates atrazine treatment did not alter the number of animals in repetitive pseudopregnancy/persistent diestrous. Estrous cycling in atrazine treated F-344 females was not altered.

Mammary Gland Histomorphology: Animals in all dose groups displayed mammary gland histomorphologic alterations that would be expected in a normally aging F-344 females rats *i.e.* - there was some evidence of lobular/acinar development with secretory activity and occasional galactoceles in all dose groups at the 15, 18 and 24 month timepoints.

The mammary gland histomorphologic alterations that are seen are those that would be expected in a normally aging F-344 female rat. Atrazine exposure did not increase the severity of any mammary gland histomorphology finding or decrease their time of onset.

4.9.4 NHEERL Publications

Substantial research has been conducted on the toxicologic effects of atrazine exposure at the Reproductive Toxicology Division of EPA's National Heath and Environmental Effects Research Laboratories (NHEERL) at Research Triangle Park, N.C. . These studies have been evaluated in the May 22, 2000 document that was reviewed by the June, 2000 SAP [see http://www.epa.gov/scipoly/sap/2000/june27/finalparta_atz.pdf]. This research includes studies investigating the neuroendocrine basis of the mode of action for atrazine-associated carcinogenesis, as well as studies investigating developmental and reproductive effects associated with atrazine exposure. Much of these data have been published in the open literature. The abstracts from these open literature publications are shown below in chronological order of publication.

Cooper, R.L., Stoker, T.E., Goldman, J.M., Parrish, M.B., Tyrey, L. (1996) Effect of atrazine on ovarian function in the rat. *Reprod. Toxicol.* 1996 Jul-Aug; 10(4):257-64. MRID not yet assigned.

The effect of the chlorotriazine herbicide, atrazine, on ovarian function was studied in Long-Evans hooded (LE-hooded) and Sprague-Dawley (SD) rats. Atrazine was administered by gavage for 21 d to females displaying regular 4-d estrous cycles. In both strains, 75 mg/kg/d disrupted the 4-d ovarian cycle; however, no distinct alteration (*i.e.*, irregular cycles but not persistent estrus or diestrus) was apparent at this dose. At 150 mg/kg/d, atrazine induced repetitive pseudopregnancies in females of both strains. The highest dose tested (300 mg/kg/d) also induced repetitive pseudopregnancies in the SD females, while the ovaries of the LE-hooded female appeared regressed and the smear cytology was indicative of the anestrous condition. Although a NOAEL was not established, the doses employed in this experiment were in excess of those used in chronic feeding studies in which an early onset of mammary gland tumors was noted. These data demonstrate that atrazine can disrupt ovarian function and bring about major changes in the endocrine profile of the female.

Stoker, T.E., Robinette, C.L., Cooper, R.L. (1999) Maternal exposure to atrazine during lactation suppresses suckling-induced prolactin release and results in prostatitis in the adult offspring. Toxicol Sci 1999 Nov; 52(1):68-79 MRID 45166902

The availability of prolactin (PRL) to the neonatal brain is known to affect the development of the tuberoinfundibular (TIDA) neurons and, as a consequence, lead to alterations in subsequent PRL regulation. Without early lactational exposure to PRL (derived from the dam's milk), TIDA neuronal growth is impaired and elevated PRL levels are present in the prepubertal male. These observations, combined with the finding that alterations in PRL secretion (*i.e.*, hyperprolactinemia) in the adult male rat have been implicated in the development of prostatitis, led us to hypothesize that early lactational exposure to agents that suppress suckling-induced PRL release would lead to a disruption in TIDA development, altered PRL regulation, and subsequent prostatitis in the male offspring. To test this hypothesis, suckling-induced PRL release was measured in Wistar dams treated twice daily with the herbicide atrazine (ATR, by gavage, on postnatal day (PND) 1-4 at 0, 6.25, 12.5, 25, and 50 mg/kg body weight), or twice daily with the dopamine receptor agonist bromocriptine (BROM, sc, at 0.052, 0.104, 0.208, and 0.417 mg/kg); BROM is known to suppress PRL release. Similarly, atrazine has also been reported to suppress PRL in adult females. Serum PRL was measured on PND 3 using a serial sampling technique and indwelling cardiac catheters. A significant rise in serum PRL release was noted in all control females within 10 min of the initiation of suckling. Fifty-mg/kg ATR inhibited suckling-induced PRL release in all females, whereas 25 and 12.5 mg/kg ATR inhibited this measure in some dams and had no discernible effect in others. The 6.25 mg/kg dose of ATR was without effect. BROM, used here as a positive control, also inhibited suckling-induced PRL release at doses of 0.104 to 0.417 mg/kg, with no effect at 0.052 mg/kg. To examine the effect of postnatal ATR and BROM on the incidence and severity of inflammation (INF) of the lateral prostate of the offspring, adult males were examined at 90 and 120 days. While no effect was noted at 90 days of age, at 120 days, both the incidence and severity of prostate inflammation was increased in those offspring of ATR-treated dams (25 and 50 mg/kg). The 12.5 mg/kg ATR and the two highest doses of BROM increased the incidence, but not the severity, of prostatitis. Combined treatment of ovine prolactin (oPRL) and 25 or 50 mg/kg ATR on PND 1-4 reduced the incidence of inflammation observed at 120 days, indicating that this increase in INF, seen after ATR alone, resulted from the suppression of PRL in the dam.

To determine whether there is a critical period for these effects, dams were dosed with 25 and 50 mg/kg on PND 6-9 and PND 11-14. Inflammation was increased in those offspring from dams treated on PND 6-9, but this increase was not significant. Dosing on PND 11-14 was without effect. These data demonstrate that ATR suppresses suckling-induced PRL release and that this suppression results in lateral prostate inflammation in the offspring. The critical period for this effect is PND 1-9.

Shafer, T.J., Ward, T.R., Meacham, C.A., Cooper, R.L. (1999) Effects of the chlorotriazine herbicide, cyanazine, on GABA(A) receptors in cortical tissue from rat brain. Toxicology 1999 Dec 20;142(1):57-68. MRID not yet assigned.

Chlorotriazine herbicides disrupt luteinizing hormone (LH) release in female rats following in vivo exposure. Although the mechanism of action is unknown, significant evidence suggests that inhibition of LH release by chlorotriazines may be mediated by effects in the central nervous system. GABA(A) receptors are important for neuronal regulation of gonadotropin releasing hormone and LH release. The ability of chlorotriazine herbicides to interact with GABA(A) receptors was examined by measuring their effects on [3H]muscimol, [3H]Ro15-4513 and [35S]tert-butylbicyclophosphorothionate (TBPS) binding to rat cortical membranes. Cyanazine (1-400 microM) inhibited [3H]Ro15-4513 binding with an IC50 of approximately 105 microM (n=4). Atrazine (1-400 microM) also inhibited [3H]Ro15-4513 binding, but was less potent than cyanazine (IC50 = 305 microM). However, the chlorotriazine metabolites diaminochlorotriazine, 2-amino-4-chloro-6-ethylamino-s-triazine and 2-amino-4-chloro-6-isopropylamino-s-triazine were without significant effect on [3H]Ro15-4513 binding. Cyanazine and the other chlorotriazines were without effect on [3H]muscimol or [35S]TBPS binding. To examine whether cyanazine altered GABA(A) receptor function, GABA-stimulated 36Cl- flux into synaptoneurosomes was examined. Cyanazine (50-100 microM) alone did not significantly decrease GABA-stimulated 36Cl- flux. Diazepam (10 microM) and pentobarbital (100 microM) potentiated GABA-stimulated 36Cl- flux to 126 and 166% of control, respectively. At concentrations of 50 and 100 microM, cyanazine decreased potentiation by diazepam to 112 and 97% of control, respectively, and decreased potentiation by pentobarbital to 158 and 137% of control (n = 6). Interestingly, at lower concentrations (5 microM), cyanazine shifted the EC50 for GABA-stimulated 36Cl- flux into synaptoneurosomes from 28.9 to 19.4 microM, respectively (n = 5). These results suggest that cyanazine modulates benzodiazepine, but not the muscimol (GABA receptor site) or TBPS (Cl- channel), binding sites on GABA(A) receptors. Furthermore, at low concentrations, cyanazine may slightly enhance function of GABA(A) receptors, but at higher concentrations, cyanazine antagonizes GABA(A) receptor function and in particular antagonizes the positive modulatory effects of diazepam and pentobarbital.

Cooper, R.L., Stoker, T.E., Tyrey, L., Goldman, J.M., McElroy, W.K. (2000) Atrazine disrupts the hypothalamic control of pituitary-ovarian function. Toxicol Sci 2000 Feb; 53(2):297-307. MRID 45166901

The chloro-S-triazine herbicides (*i.e.*, atrazine, simazine, cyanazine) constitute the largest group of herbicides sold in the United States. Despite their extensive usage, relatively little is known about the possible human-health effects and mechanism(s) of action of these compounds. Previous studies in our laboratory have shown that the chlorotriazines disrupt the hormonal control of ovarian cycles. Results from these studies led us to hypothesize that these herbicides disrupt endocrine function primarily through their action on the central nervous system. To evaluate this hypothesis, we examined the estrogen-induced surges of luteinizing hormone (LH) and prolactin in ovariectomized Sprague-Dawley (SD) and Long-Evans hooded (LE) rats treated with atrazine (50-300 mg/kg/day, by gavage) for 1, 3, or 21 days. One dose of atrazine (300 mg/kg) suppressed the LH and prolactin surge in ovariectomized LE, but not SD female rats. Atrazine (300 mg/kg) administered to intact LE females on the day of vaginal proestrus was without effect on ovulation but did induce a pseudopregnancy in 7 of 9 females. Three daily doses of atrazine suppressed the estrogen-induced LH and prolactin surges in ovariectomized LE females in a dose-dependent manner, but this same treatment was without effect on serum LH and prolactin in SD females. The estrogen-induced surges of both pituitary hormones were suppressed by atrazine (75-300 mg/kg/day) in a dose-dependent manner in females of both strains evaluated after 21 days of treatment. Three experiments were then performed to determine whether the brain, pituitary, or both organs were the target sites for the chlorotriazines. These included examination of the ability of (1) the pituitary lactotrophs to secrete prolactin, using hypophysectomized females bearing pituitary autotransplants (ectopic pituitaries); (2) the synthetic gonadotropin-releasing hormone (GnRH) to induce LH secretion in females treated with high concentrations of atrazine for 3 days; and (3) atrazine (administered in vivo or in vitro) to suppress LH and prolactin secretion from pituitaries, using a flow-through perfusion procedure. In conclusion, the results of these studies demonstrate that atrazine alters LH and prolactin serum levels in the LE and SD female rats by altering the hypothalamic control of these hormones. In this regard, the LE female appeared to be more sensitive to the hormone suppressive effects of atrazine, as indicated by the decreases observed on treatment-day 3. These experiments support the hypothesis that the effect of atrazine on LH and prolactin secretion is mediated via a hypothalamic site of action.

Das, P.C., McElroy, W.K., Cooper, R.L. (2000) Differential modulation of catecholamines by chlorotriazine herbicides in pheochromocytoma (PC12) cells in vitro. Tox. Sci. Aug; 56(2): 324-31. MRID not yet assigned.

Epidemiological, wildlife, and laboratory studies have pointed to the possible adverse health effects of chlorotriazine herbicide (*i.e.* , atrazine, simazine, and cyanazine) exposure. However, the cellular mechanism(s) of action of these compounds remains unknown. Recently, it was reported by Cooper et al. (2000, Toxicol. Sci. 53, 297-307) that atrazine disrupts ovarian function by altering hypothalamic catecholamine concentrations and subsequently the regulation of luteinizing hormone (LH) and prolactin (PRL) secretion by the pituitary. In this study, we examined the effect of three chlorotriazines on catecholamine metabolism in vitro using PC12 cells. Intracellular norepinephrine (NE) and dopamine (DA) concentrations and spontaneous NE release were measured following treatment with different concentrations of atrazine, simazine (0, 12. 5, 25, 50, 100, and 200 microM) and cyanazine (0, 25, 50, 100, and 400 microM) for 6, 12, 18, 24, and 48 h. Atrazine and simazine significantly decreased intracellular DA concentration in a concentration-dependent manner. Intracellular NE concentration was also significantly decreased by 100 and 200 microM atrazine and 200 microM simazine. Similarly, there was a dose-dependent inhibition of NE release with 100 and 200 microM concentrations of both compounds. Although 100 and 400 microM cyanazine increased intracellular NE concentration, 50, 100, and 400 microM cyanazine significantly increased NE release at 24 and 36 h. In contrast, intracellular DA concentration was decreased by cyanazine, but only at 400 microM. The GABA(A)-receptor agonist, muscimol (0, 0.01, 0.1, and 1.0 microM) had no effect on either the release or on intracellular catecholamine concentrations from 6 through 24 h of treatment. Cell viability was somewhat lower in the groups exposed to 100 and 200 microM atrazine and simazine. However, the reduction in viability was significant only in the highest dose of atrazine used (200 microM) at 24 h. Cyanazine did not have an effect on the viability at any of the doses tested, and the cells were functional, even up to 48 h of exposure. These data indicate that both atrazine and simazine inhibit the cellular synthesis of DA mediated by the tyrosine hydroxylase (TH), and NE mediated by dopamine beta-hydroxylase (DbetaH), and, as a result, there is a partial or significant inhibition of NE release. Cyanazine, on the other hand, stimulated the synthesis of intracellular NE, and not DA. Thus, chlorotriazine compounds presumably act at the enzymatic steps or sites of DA biosynthesis to modulate monoaminergic activity in PC12 cells.

Cummings, A. M., Rhodes, B.E., and Cooper, R.L. (2000). Effect of atrazine on implantation and early pregnancy in four strains of rats. *Tox. Sci.* Nov. 58: 135-143. MRID not yet assigned.

Atrazine (ATR) is an herbicide that has been shown to have adverse reproductive effects including alterations in levels of pituitary hormones such as prolactin (prl) and luteinizing hormone (LH) in female LE rats when administered at doses of 200 mg/kg/day for 1 and 3 days. Since prl's action to promote progesterone secretion is essential for the initiation of pregnancy in rats, this study was designed to examine the effect of exposure to ATR during early pregnancy on implantation and short-term pregnancy maintenance. Rats were divided into two groups representing periods of dosing with ATR prior to the diurnal or nocturnal surges of prl. Within each group, four groups consisting of four strains of rats (Holtzman, HLZ; Sprague Dawley, SD; Long Evans, LE; Fisher 344, F344) were each further subdivided into four ATR dosages. Rats were dosed by gavage with 0, 50, 100, or 200 mg/kg/day ATR on days 1-8 of pregnancy (day 0 = sperm +). All animals were necropsied on day 8 or 9 of pregnancy. The 200 mg/kg dose of ATR reduced body weight gain in all but one group. Two groups of animals dosed at 100 and 200 mg/kg/day in the nocturnal dosing period showed an increase in percent preimplantation loss, and both of these were F344 rats. Holtzman rats were the only strain to show a significant level of postimplantation loss and a decrease in serum progesterone at 200 mg/kg/day both following diurnal and nocturnal dosing. Doses of 100 mg/kg/day also produced postimplantation loss following diurnal and nocturnal dosing, but progesterone levels were only decreased after nocturnal dosing. Alterations in serum LH were seen in several groups. Serum estradiol was significantly increased only in Sprague Dawley rats dosed at the diurnal interval with 200 mg/kg ATR. We conclude that F344 rats are most susceptible to preimplantation effects of ATR and that HLZ rats appear most sensitive to the postimplantation effects of the chemical. LE and SD rats were least sensitive to effects of ATR during very early pregnancy.

Narotsky M. G., Best, D.S., Guidici, D. L., and Cooper, R.L. (2000). Strain comparisons of atrazine-induced pregnancy loss in the rat. *Repro Toxicol.* In Press. MRID not yet assigned.

Atrazine was administered by gavage, in 1% methylcellulose, to F344 Sprague-Dawley (SD), and Long Evans (LE) rats at 0, 25, 50, 100, or 200 mg/kg/d on gestation days 6 through 10. The dams were allowed to deliver and litters were examined postnatally. The F344 strain was the most sensitive to atrazine's effects on pregnancy, showing full-litter resorption (FLR) at $50 mg/kg. In surviving F344 litters, prenatal loss was increased at 200 mg/kg. In SD and LE rats, FLR occurred only at 200 mg/kg. Delayed parturition was seen at ≥100 mg/kg in F344 and SD rats. Regarding maternal toxicity, the SD dams were the most sensitive, with weight loss at ≥25 mg/kg. When 200 mg/kg was administered to F344 rats on days 11 through 15 (after the LH-dependent period of pregnancy), no FLR was seen. These findings suggest that atrazine-induced FLR is maternally mediated, and consistent with loss of LH support of the corpora lutea.

> Laws, S.C., Ferrell, J.M., Stoker, T.E., Schmid, J., and Cooper, R.L. (2000). The effect of atrazine on puberty in female Wistar rats: an evaluation in the protocol for the assessment of pubertal development and thyroid function. *Tox. Sci.* 58 (2):366-76. MRID not yet assigned.

The effects of atrazine (ATR), a chlorotriazine herbicide, on the onset of puberty were evaluated in Wistar rats. Female rats were dosed by oral gavage from postnatal day (PND) 22 through PND 41 with 0, 12.5, 25, 50, 100 or 200 mg ATR /kg. Vaginal opening (VO) was significantly delayed 3.4, 4.5 or greater than 6.8 days by 50, 100 and 200 mg/kg, respectively. VO did not occur in 4 of 15 females in the 200 mg/kg group by the time of necropsy (PND 41). Body weight (BWT) at necropsy was reduced in the 200 mg/kg group by 11.6%, but was not different from the control (0) in the 50 and 100 mg/kg groups. To examine the influence of reduced BWT on pubertal development, a group of pair-fed controls was included whose daily food intake was dependent upon the amount consumed by their counterpart in the 200 mg/kg group. Although necropsy BWT was reduced to the same extent as the ATR females, VO in the pair-fed controls was not significantly delayed. Adrenal, kidney, pituitary, ovary and uterine weights were reduced by 200 mg/kg ATR. Serum T3, T4 and TSH were unaltered by ATR which were consistent with no histopathologic/morphologic changes in the thyroid. Estrous cyclicity was monitored in a second group of females from VO - PND 149. The number of females displaying regular 4 or 5-day estrous cycles during the first 15-day interval after VO, was lower in the 100 and 200 mg/kg ATR and pair-fed controls. Irregular cycles were characterized by extended periods of diestrus. By the end of the second 15-day interval (PND 57-71), no effects on estrous cyclicity were observed. These data show that ATR can delay the onset of puberty and alter estrous cyclicity in the female Wistar rat (NOAEL of 25 mg/kg). Reduced food consumption and BWT did not account for the delay in VO because this effect was not observed in the pair-fed controls. In addition, the effect on estrous cyclicity was observed in the 100 mg/kg ATR group where no significant reduction in BWT was observed.

> Stoker, T.E., Laws, S.C., Guidici, D. and Cooper, R.L. (2000) The effect of atrazine on puberty in male Wistar rats: An evaluation in the protocol for the assessment of pubertal development and thyroid function. *Toxicol. Sci.* Nov. 58: 50-59.

Since atrazine (ATR), a chlorotriazine herbicide, has been shown previously to alter the secretion of luteinizing hormone (LH) and prolactin (PRL) through a direct effect on the CNS, we hypothesized that exposure to ATR in the EDSTAC male pubertal protocol (juvenile to peripubertal) would alter the development of the male rat reproductive system. We dosed intact male Wistar rats from postnatal day (PND) 23 to 53 and examined several reproductive endpoints. ATR (0, 12.5, 25, 50, 100, 150 or 200 mg/kg) was administered by gavage and an additional pair-fed group was added to compare the effects of any decreased food consumption in the high dose group. Preputial separation (PPS) was significantly delayed in the 12.5, 50, 100, 150 and 200 mg/kg ATR dose groups. PPS was also delayed in the pair-fed group, although significantly less than in the high dose ATR group. The males were killed on PND 53 or 54 and pituitary, thyroid, testes, epididymides, seminal vesicles, ventral and lateral prostates were removed. ATR (50 to 200 mg/kg) treatment resulted in a significant reduction in ventral prostate weights, as did the pair-fed group. Testes weights were unaffected by atrazine treatment. Seminal vesicle and epididymal weights were decreased in the high dose ATR group and the control pair-fed group. However, the difference in epididymal weights was no longer significantly different when body weight was entered as a covariable. Intratesticular testosterone was significantly decreased in the high dose ATR group on PND 45, but apparent decreases in serum testosterone were not statistically significantly on PND 53, There was a trend for a decrease in luteinizing hormone (LH) as the dose of ATR increased, however, dose group mean LH were not different from controls. Due to the variability of serum prolactin concentrations on PND 53, no significant difference was identified. Although prolactin is involved in the maintenance of LH receptors prior to puberty, we observed no difference in LH receptor number at PND 45 or 53. Serum estrone and estradiol showed dose-related increases that were significant only in the 200 mg/kg ATR group. No differences were observed in thyroid stimulating hormone (TSH) and thyroxine (T4) between the ATR groups and the control, however tri-iodothyronine (T3) was elevated in the high dose ATR group. No differences in hormone levels were observed in the pair-fed animals. These results indicate that ATR delays puberty in the male rat and its mode of action appears to be altering the secretion of steroids and subsequent effects on the development of the reproductive tract, which appear to be due to ATR's effects on the CNS. Thus, ATR tested positive in the pubertal male screen that EDSTAC is considering as an optional screen for endocrine disrupters.

4.10 Toxicology data for major metabolites of atrazine

Metabolism of atrazine can occur through hydrolytic dehalogenation at the 2 position of the s-triazine ring resulting in the formation of hydroxyatrazine. Dealkylation reactions at the 4 and 6 positions - either in the absence or presence of dehalogenation- may also occur. Both plants and animals are capable of performing the dealkylation reactions. Hydroxyatrazine is the major metabolite in plants, but is only a minor metabolite in animals where the varying forms of the delkylated chlorometabolites dominate instead. The metabolism of atrazine to hydroxyatrazine in plants is a detoxification reaction as the phytotoxicity of hydroxyatrazine is greatly reduced compared to the parent compound. Bacteria are believed to be capable of the dealkylation reactions that form the chlorometabolites of atrazine. Hydroxyatrazine and the chlorometabolites can be found in both drinking water and the diet.

The HED Metabolism Committee (October 17, 2000) has determined that for chronic dietary risk, the residues of concern are parent and chlorometabolites compared to appropriate atrazine endpoints. A separate chronic dietary risk assessment will be conducted for hydroxyatrazine metabolites compared to the appropriate hydroxyatrazine endpoint. For acute dietary risk assessment the residues of concern are the parent chlorometabolites compared to appropriate acute atrazine endpoints. A separate acute assessment will not be conducted for hydroxyatrazine as HIARC did not select an acute RfD for hydroxyatrazine. The three chlorometabolites of concern are diaminochlorotriazine, desisopropyl atrazine , and desethyl atrazine. A limited toxicology database is available for these three metabolites and these studies are summarized below in sections 4.10.1, 4.10.2, and 4.10.3. A limited toxicology database is also available for hydroxyatrazine and is shown below under section 4.10.4.

4.10.1 Diaminochlorotriazine metabolite (DACT) - 2-chloro-4-amino-6-amino s-triazine;

dideakyl atrazine; G-28273

The DACT metabolite is the terminal mammalian metabolite of both atrazine and the closely related herbicide simazine. DACT is primarily a mammalian metabolite but can also be produced in plants and bacteria. DACT is produced through dealkylation of the isopropyl group at position 2 of the triazine ring in atrazine (dealkylation of the ethyl group at the 2 position of the triazine ring in simazine) and dealkylation of the ethyl group at the 4 position of the triazine ring in either atrazine or simazine. DACT is the primary metabolite produced in rats following atrazine treatment. It is the major fecal metabolite in rats accounting for 40% of the radioactivity found in feces following exposure by oral gavage. DACT is also the major urinary metabolite in rats accounting for 26% of the total administered dose following exposure by oral gavage.

870.3100 - Subchronic oral toxicity in rats

Groups of 15 male and 15 female CD Sprague-Dawley rats were fed diets containing diaminochlorotriazine (G-28273) (purity 98.2%) at concentrations of 0, 10, 100, 250, or 500 ppm for 13 weeks (MRID 43013207). The average consumption of test material was 0.7, 6.7, 16.7, or 34.1 mg/kg/day (males) and 0.7, 7.6, 19.7, or 40.2 mg/kg/day (females). All animals survived to study termination. No treatment-related clinical signs of toxicity including ocular lesions were seen at any dose level. At 500 ppm, mean body weights of male rats were lower than controls during most of the study period, decreasing to 87% of controls at week 12. Body weight gain at week 12 was 82% of controls for males receiving 500 ppm, and 85% and 83% of controls for females receiving 250 and 500 ppm, respectively. No treatment-related effects on body weight or body weight gain were seen at the lower doses in either sex. Food consumption was not affected by administration of the test material. There were no biologically significant effects on hematology, clinical chemistry, urinalysis, and gross or histopathology at any dose level. Although several organ weight changes were observed, there were no histologic or functional correlates. Estrous cycle data indicated a treatment-related effect at doses of $100 ppm. The effects, generally more pronounced on days 70-85 than on days 14-28 and 42-56, included lengthening of the estrus cycle and/or an increased incidence of rats exhibiting cycles with persistent estrus and/or diestrus. There were no apparent effects on serum levels of estradiol, progesterone, prolactin, and corticosterone.

The LOAEL is 7.6 mg/kg/day based on estrous cycle effects in female rats. The NOAEL is 0.7 mg/kg/day.

Classification: This study is classified as **Acceptable-Guideline** and satisfies the guideline requirements for a subchronic dietary toxicity study (870.3100) in rats.

870.3150 and 870.4100 - Subchronic and chronic oral toxicity in dogs

Diaminochlorotriazine was fed to male and female dogs at dietary levels of 0,5,100, or 1500 ppm for 13 or 52 weeks (MRID 41392401). Because of severe toxicity at the highest dose, which was evident after 6 weeks of treatment, the high-dose dogs were fed a diet containing 750 ppm. Females tolerated this dose level and received 750 ppm until termination at 13 or 52 weeks, or through 13 weeks followed by a 39-week recovery period. Since males continued to exhibit signs of toxicity at 750 ppm, they were fed untreated diet for 9 weeks through 13. Four male dogs were then placed again on a diet containing 750 ppm until termination at 52 weeks.

The mean daily doses for male dogs receiving dietary levels of 5, 100, and 1500/750 ppm for 52 weeks were 0.187, 3.61, and 24.1 mg/kg/day, While the doses were 0.195, 3.43, and 32.7 mg/kg/day for females receiving the same dietary levels. Among the high-dose dogs, five males and two females were sacrificed moribund during the treatment period. Moribundity was attributed to impairment of heart function, the primary treatment-related effect of Diaminochlorotriazine, which was accompanied by several clinical and pathological changes. Pathological cardiac findings included enlargement, softness, thickened valves, lesions, distension, red/dark color, thrombosis, chronic myocarditis, necrosis, inflammation, hemorrhage, and hemosiderosis. Secondary treatment-related changes in the high-dose animals were seen in the liver (enlargement, congestion, centrilobular fibrosis/atrophy, bile stasis, necrosis, hemosiderosis, red/dark color, lesions, adhesions, mottling, and rough texture); testes (hypospermatogenesis and hypospermia); thymus (atrophy); bone marrow (hyperplasia); and pericardium, thoracic, and abdominal cavities (fluid accumulation). Recovery females did not exhibit any clinical ophthalmological signs of cardiac impairment. Other effects at the high dose included decreased body weight gains in males and females dosed for 6 weeks; increased mean spleen, liver, and kidney weights; anemia with accompanying reticulocytosis (a reversible effect); decreases in albumin, calcium and total cholesterol levels; nonsignificant increase in lactic acid dehydrogenase activity ; and elevations of platelet levels. High-dose males continued to lose weight when the dose was lowered to 750 ppm. Severe anemia with reticulocytosis was noted in only one of the two recovery females. The effects of diaminochlorotriazine on cholesterol levels and erythroid parameters were reversible as were noted in almost all animals of both sexes and beginning at week 6 and extending until week 14. No adverse effects were observed at dietary levels of 5 or 100 ppm. Although there was appreciable mortality at the highest dose of 1500 ppm, a sufficient number of animal were at risk to evaluate histopathology. Administration of dietary levels of $ 750 ppm to dogs is associated with symptomatology of cardiac impairment.

The LOAEL is 24.1 mg/kg/day based on cardiac effects. The NOAEL is 3.43 mg/kg/day.

This study is classified as **Acceptable-Guideline** and satisfies the guideline requirements for a subchronic dietary toxicity study (870.3150) and also a chronic dietary toxicity study (870.4100) in dogs.

870.3700 - Developmental toxicity in rats

In a developmental toxicity study (MRID 41392402) diaminochlorotriazine, 98.2 % a.i. was administered to 130 Sprague-Dawley females, 26/dose, by gavage at dose levels of 0, 2.5 , 25, 75 or 150 mg/kg/day from days 6 through 16 of gestation. Animals were sacrificed and uteri removed for evaluation on gestation day 20.

Maternal body weight gain for the 20 day gestation period was reduced 33.2% (p# 0.05), compared to controls, at 150 mg/kg/day. Body weight gain during the dosing period was reduced 27.9% (not significant) and 71% (p# 0.05) at the 75 and 150 mg/kg/day groups, respectively, compared to controls. Food consumption for the 20 day gestation was reduced by 21.1% (p# 0.05), compared to controls, at 150 mg/kg/day. Body weight gains and food consumption were not affected at any other dose group compared to control. There were no treatment-related effects in mortality or clinical signs in any dose group.

The maternal LOAEL is 75 mg/kg/day, based on decreased body weight gain during dosing. The maternal NOAEL is 25 mg/kg/day.

Resorptions per dam were increased from 0.8 in controls to 2.6 (p# 0.05) in the 150 mg/kg/day group. Postimplantation loss was increased from 5.6% in controls to 18.9% (p# 0.05)in the 150 mg/kg/day group. Gravid uterine weight per dam was decreased from 72.3 grams in the controls to 55.9 grams (p# 0.05) at 150 mg/kg/day. Mean fetal weights were reduced 19.1% and 18.5% in males and females, respectively, at the 150 mg/kg/day dose group compared to controls (p# 0.05 for both). Mean fetal weights were reduced 9% and 7.9% for males and females, respectively at the 75 mg/kg/day group (p# 0.05 for both). Absent renal papilla were seen in 22% of the 150 mg/kg/day fetuses compared to 3.3% of the control fetuses (p# 0.05). Pitted kidneys were seen in 4.8% of the 150 mg/kg/day fetuses and in 0% of the control fetuses (p# 0.05). When evaluated using either the fetus or the litter as the experimental unit, skeletal examinations revealed statistically significant (p# 0.05) increases in incomplete ossification of several bones in both the 75 and 150 mg/kg/day dose groups compared to controls. At 25 mg/kg/day there were significant (p# 0.05) increases, compared to controls, of fetuses with incompletely ossification of these three bones only: interparietals (18.1% *vs* 35%); incompletely ossified parietals (3.4% *vs* 10.6%); and, unossified hyoids (4.7% *vs* 15.3%). The increases were also significant (p# 0.05) at 25 mg/kg/day for these three findings when the litter was used as the experimental unit.

The developmental LOAEL is 25 mg/kg/day, based on increases in incidences of incompletely ossified parietals, interparietals and unossified hyoids. The developmental NOAEL is 2.5 mg/kg/day.

The developmental toxicity study in the rat is classified **Acceptable-Guideline** and <u>does</u> satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700) in the rat.

870.5100 Bacterial Reverse Mutation

In replicate (separate) trials, the atrazine metabolite diaminochlorotriazine (DACT, 97.0%) failed to induce reverse mutation (as evidenced by no increase over controls in revertant colony counts) in Ames testing at concentrations up to 5000 :g/0.1 mL, at which dose precipitation of compound occurred. This study is classified **Acceptable-Guideline** (MRID 40722302).

870.5550 UDS Assay

The atrazine metabolite diaminochlorotriazine (DACT, 97.0%) did not induce unscheduled DNA synthesis in cells of the human fibroblast cell CRL 1521 exposed to concentrations resulting in compound precipitation (400 to 600 :g/mL), but only in the absence of a metabolic activation system (assay without activation was not performed). This study is classified **Acceptable-nonguideline** (MRID 40722303).

4.10.2 G-28279 metabolite -2-chloro-4-(ethylamino)-6-(amino) s-triazine; ethylatrazine; deisopropyl atrazine

Desisopropyl atrazine is a metabolite of both atrazine and simazine. Desisopropyl atrazine is primarily a mammalian metabolite but can also be produced in plants and bacteria. Dealkylation of the isopropyl group from the 2 position of the triazine ring in atrazine or simazine yields desisopropyl atrazine/simazine.

870.3100 - Subchronic oral toxicity in rats

In a 90 day study (MRID 43013205) groups of 10 male and 10 female RAIf(SPF) rats were fed diets containing G-28279 technical (purity 96.7%; Lot #FL-901747) at concentrations of 0, 10, 50, or 500 ppm for 13 weeks. The average consumption of test material was 0.602, 3.20, or 34.9 mg/kg/day (males) and 0.641, 3.34, or 37.5 mg/kg (females). All animals survived to study termination. No treatment-related clinical signs or ocular lesions were seen at any dose level. At 10 ppm, there were no effects on body weight, clinical chemistry parameters, hematologic values, or organ weights, nor were macroscopic or histopathologic changes observed. Slightly decreased body weights and body weight gains were seen in males administered 50 or 500 ppm of the test material. At week 13, the body weights of males were 93% and 88% of control values at 50 and 500 ppm, respectively, and the body weight gains were 96, 90, or 84% of control values at 10, 50, or 500 ppm, respectively. For female rats exposed to 500 ppm, the body weights were 9-12% below those of controls from treatment week 2 to 13, and the mean body weight gain was 80% of controls at the end of the treatment period. Food consumption was not significantly affected by treatment with G-28279 technical. Organ weight changes (absolute and relative to body weight) in males at 500 ppm included increased relative kidney weights (14% above control values), decreased absolute heart weights (-10%), increased relative testes weights (+21%), and increased relative brain weights (+18%). In the absence of histopathologic lesions in the corresponding organs, these organ weight changes are of uncertain toxicological significance and may not be treatment-related. Increased relative liver weights (+13%) and extramedullary hematopoiesis of the liver seen in female rats at 500 ppm indicate slight hepatotoxicity. Minimal to moderate fatty changes of adrenal cortex and slight hypertrophy of the thyroid follicular epithelium in males at 500 ppm suggest treatment-related effects. Additionally, extramedullary hematopoiesis of the spleen in females at 500 ppm and hypertrophy of pituitary cells in males at 500 ppm may suggest treatment related effects.

The LOAEL is 3.3 mg/kg/day, based on decreased body weights and body weight gains in both sexes. The corresponding NOAEL is 0.6 mg/kg/day.

Classification: This study is classified as **Acceptable-Guideline** and satisfies the guideline requirements for a subchronic dietary toxicity study (870.3100) in rats.

870.3150 - Subchronic oral toxicity in dogs

In a subchronic study (MRID 43013203) male and female beagle dogs (four/sex/group) were fed diets containing 0,15,100, 500, or 1000 ppm G-28279 technical for at least 14 weeks (equivalent to compound intakes of 0, 0.6, 3.8, 18.9, and 33.4 mg/kg/day for male dogs, and 0.6, 3.8, 18.0, and 33.3 mg/kg/day for female dogs).

Male dogs in the 1000-ppm and female dogs in the 500-ppm and 1000-ppm groups exhibited significant decreases in food consumption and decreased body weight gain relative to controls and to respective baseline values. Mean food efficiency was negative in male dogs of the 1000-ppm and female dogs of the 500-ppm and 1000-ppm groups. Clinical chemistry, hematology, and urinalysis data showed alterations that were of sporadic or intermittent occurrence, were not dose-related, and were not sustained for any period of time. Gross and histopathological examinations revealed no treatment-related findings. A statistically significant decrease in mean absolute and mean relative (to brain) heart weight were observed for the 500-ppm and 1000-ppm male dogs but there were no histopathologic or functional (EKG) correlates indicating myocardial toxicity. Although the study authors attributed the alterations in heart weight to be secondary to inanition, the significant decrease (17% in the 500-ppm group, $0.01<p<0.05$; 25% in the 1000-ppm group, p#0.01) in mean relative (to brain) heart weight may be indicative of a treatment-related effect. Similar findings were noted for organ-to-brain weight values for testes and prostate glands in the 500-ppm and 1000-ppm dogs. Two dogs (litter mates) were found to be anemic but the condition was not considered to be related to test article exposure. Although most of the observed effects may be secondary to inanition, the decreased organ-to brain weight ratios for the heart, testes, and prostate gland, and the negative food efficiency observed in dogs exposed to dietary G-28279 at concentrations of 500 or 1000 ppm are indicative of marginally toxic effects.

The LOAEL is 18.9 mg/kg/day based on decreased food consumption, body weight and heart weight (relative to brain weight). The NOAEL is 3.8 mg/kg/day. .

This study is classified as **Acceptable-Guideline** and satisfies the guideline requirements for a subchronic feeding study (870.3150) in dogs.

870.3700 - Developmental toxicity in rats

In a developmental toxicity study (MRID 43013208), 24 Tif: RAI rats per group received G-28279 technical by gavage on gestational days (GDs) 6-15, inclusive, at dose levels of 0, 5, 25, or 100 mg/kg/day. Aqueous corn starch (3%) served as the control substance and vehicle for the test mixture.

Maternal toxicity was observed at 25 and 100 mg/kg/day in a dose-related manner as evidenced by effects on body weight/weight gain and food consumption. Body weights were significantly decreased at 25 mg/kg/day on GDs 9 and 13 (96% of control) and at 100 mg/kg/day on GD 7 through GD 18 (93% of control). Weight gains were decreased on GDs 6-11 at 25 mg/kg/day (73% of control, nonsignificant) and at 100 mg/kg/day (30% of control, significant) and on GDs 11-16 at 100 mg/kg/day (89% of control, nonsignificant). Corrected weight gain was significantly decreased at 100 mg/kg/day (55% of control). Food consumption was significantly decreased on GDs 6-11 at 25 mg/kg/day (91% of control) and at 100 mg/kg/day (70% of control).

The maternal toxicity LOAEL is 25 mg/kg/day based on decreased body weight and food consumption. The NOAEL is 5 mg/kg/day.

Developmental toxicity was observed at 25 and 100 mg/kg/day as evidenced by effects on skeletal development. Fetal and litter incidences of fused sternebrae 1 and 2 were significantly increased at both these dose levels, while fetal incidences only at 100 mg/kg/day of poor ossification in sternebrae 2 and absent ossification in several digits were also increased significantly.

The developmental toxicity is 25 mg/kg/day based on increased fetal and litter incidences of fused sternebrae. The NOAEL is 5 mg/kg/day.

This study is classified as **Acceptable-Guideline** and satisfies the guideline requirements for a developmental study (870.3700) in rats.

870.5100 Bacterial Reverse Mutation Assay

The atrazine metabolite G-28279 (97.4%) was negative for inducing genetic reversions at the histidine locus in the Ames battery of *Salmonella typhimurium* or tryptophan prototrophy in *Escheria coli* WP2uvrA, when tested up to the limit dose of 5000 : g/plate. This study is classified **Acceptable-Guideline** (MRID 43093101).

870.5385 Mammalian Bone Marrow Chromosome Aberration Test

The atrazine metabolite G-28279 (97.4%) was reported negative for the induction of micronuclei in polychromatic erythrocytes of Tif:MAGF mice treated orally at single doses up to clinically (but not cytologically) toxic levels (MTD = 480 mg/kg). This study is classified **Acceptable-Guideline** (43093103).

870.5550 UDS Assay

The atrazine metabolite G-28279 (97.4%) was negative for inducing unscheduled DNA synthesis in primary rat hepatocyte cultures up to cytotoxic concentrations (800 : g/mL). This study is classified **Acceptable-Guideline** (43093105).

4.10.3 G-30033 metabolite -2-chloro-4-amino-6-(isopropylamino) s-triazine; deethyl atrazine; isopropyl atrazine.

Deethyl atrazine is a metabolite of atrazine, but not simazine . Deethyl atrazine is primarily a mammalian metabolite but can also be produced in plants and bacteria. Dealkylation of the ethyl group from the 4 position of the triazine ring yields deethyl atrazine.

870.3100 - Subchronic oral toxicity in rats

In a subchronic study (MRID 43013206) G-30033 Technical, a metabolite of atrazine was given in the diet to four groups of 10 male and 10 female RAIf (SPF) rats at a concentration of 0, 10, 50, or 500 ppm for 13-weeks. The doses were equivalent to an average dose of 0.68, 3.2, and 35.1 mg/kg body weight/day or 0.72, 3.3, and 38.1 mg/kg body weight/day for male and female rats, respectively. The only treatment-related effects reported include: a decrease in the body weight of high dose female rats and a decrease in the food efficiency of high-dose male and female rats.

The LOAEL is 35.1 mg/kg/day based on decreased body weight and food efficiency. The NOAEL is 3.2 mg/kg/day.

This study is classified as **Acceptable-Guideline** and satisfies the guideline (870.3100) requirements for a subchronic oral study in rats.

870.3150 - Subchronic oral toxicity in dogs

In a subchronic study (MRID 43013203) Beagle dogs were divided into four groups, each containing four males and four females, and given diets containing 0, 15, 100, or 1000 ppm G-30033 (equivalent to 0, 0.56, 3.71, or 28.85 mg/kg/day for males and 0, 0.51, 3.88, and 32.18 mg/kg/day for females) for 13 weeks. Dietary exposure did not induce mortality, clinical signs of toxicity, ocular toxicity, or effects detectable in the urine. Treatment significantly decreased the weight gain of male and female dogs in the 1000 ppm group, however, the effect was attributed to inanition as a result of food palatability rather than an effect induced by the test material. The test material induced renal tubular hyperplasia/basophilia in 3/4 male and 2/4 female dogs in the 1000 ppm treatment group. Marginal threshold effects found in male and female dogs fed diets containing 1000 ppm test material include: decreased absolute and relative (to brain) heart weights, normocytic/normochromic anemia, paroxysmal atrial fibrillation, and right atrial wall hemorrhagic inflammation with angiomatous hyperplasia. The marginal threshold effects may be related to G-30033 treatment, however without the use of pair-fed control dogs, they can not be separated from effects induced by inanition.

The LOAEL for male and female dogs is 30 mg/kg/day based on renal tubular hyperplasia. The NOAEL is 3.8 mg/kg/day.

Classification: This study is classified as **Acceptable-Guideline** and satisfies the guideline requirements for a subchronic feeding study (870.3150) in beagle dogs.

870.3700 - Developmental toxicity in rats

In a developmental toxicity study (MRID 43013209) 24 Tif: RAI rats per group received G-30033 technical by gavage on gestational days (GDs) 6-15, inclusive, at dose levels of 0, 5, 25, or 100 mg/kg/day. Aqueous corn starch (3%) served as the control substance and vehicle for the test mixture.

Maternal toxicity was observed at 25 and 100 mg/kg/day in a dose-related manner as evidenced by effects on body weight/weight gain and food consumption. Body weights were significantly decreased at 100 mg/kg/day (94% of control) on GD 7 through GD 20. Weight gains were significantly decreased on GDs 6-11 at 25 mg/kg/day (83% of control) and at 100 mg/kg/day (41% of control) and on GDs 11-16 at 100 mg/kg/day (87% of control). Corrected weight gains were non-significantly decreased at 25 mg/kg/day (73% of control) and at 100 mg/kg/day (80% of control). Food consumption was significantly decreased on GDs 6-11 at 25 mg/kg/day (91% of control) and at 100 mg/kg/day (70% of control).

The maternal toxicity LOAEL is 25 mg/kg/day based on decreased body weight gain and food

consumption. The NOAEL is 5 mg/kg/day.

Developmental toxicity was observed at 100 mg/kg/day as evidenced by effects on skeletal development. Fetal and litter incidences of fused sternebrae 1 and 2 were significantly increased at 100 mg/kg/day and the fetal incidence of poor ossification of the proximal phalanx of posterior digit 5 (a skeletal variation) at 100 mg/kg/day was also increased significantly.

The LOAEL for developmental toxicity and 100 mg/kg/day based on increased fetal and litter incidences of fused sternebrae and increased fetal incidence of poor ossification of the proximal phalanx of posterior digit 5. The NOAEL is 25 mg/kg/day.

This study is classified as **Acceptable-Guideline** and satisfies the guideline requirements for a developmental study (870.3700) in rats.

870.5100 Bacterial Reverse Mutation Assay

The atrazine metabolite G-30033 (99.3%) was negative for inducing reverse gene mutation at the histidine locus of *Salmonella typimurium* and for the tryptophan locus in *Escheria coli* when tested to the limit dose, 5000 : g/plate. This study is classified **Acceptable-Guideline** (MRID 43093102).

870.5385 Mammalian Bone Marrow Chromosome Aberration Test

The atrazine metabolite G-30033 (99.3%) was reportedly negative for induction of micronuclei in the PCE of Tif:MAGF mice treated once orally up to clinically (but not cytologically) toxic doses (MTD = 480 mg/kg). This study is classified **Acceptable-Guideline** (MRID 43093104).

870.5550 UDS Assay

The atrazine metabolite G-30033 (99.3%) was negative for inducing unscheduled DNA synthesis in primary rat hepatocyte cultures exposed up to cytotoxic concentrations (1000 : g/mL). This study is classified **Acceptable-Guideline** (MRID 43093106).

4.10.4 Hydroxyatrazine - 2- hydroxy - 4(ethylamino)- 6(isopropylamino) - s- triazine

Hydroxyatrazine is a metabolite of both atrazine and simazine. Hydroxyatrazine is primarily a plant metabolite but can also be produced in mammals and bacteria. Hydroxyatrazine is produced by dehalogenation of the chlorine from the triazine ring of either atrazine or simazine. Dealkylation reactions preceding or following the dehalogenation allow for the production of diamino, desisopropyl, and deethyl hydroxyatrazine or hydroxysimazine.

870.3100 - Subchronic oral toxicity in rats

In a subchronic toxicity study (MRID 41293501) hydroxyatrazine (97.1%) was administered to 150 Sprague-Dawley rats, 15/sex/dose in the diet at dose levels of 0, 10, 100, 300 and 600 ppm (0, 0.64, 6.3, 18.89 and 37.47 mg/k/g/day for males and 0, 0.75, 7.35, 22.75 and 45.64 mg/kg/day for females).

With the exception of kidney alterations, most of the adverse effects of hydroxyatrazine exposure were limited to the high dose group. Body weights were non-significantly reduced in both sexes at the high dose only (7% reduction compared to controls in males and 5% reduction in females). Percent body weight gain was significantly decreased in both sexes at the high dose only (14.4% decrease compared to controls in males and 13.6% in females). Food consumption was significantly decreased in males only and only at a few selected timepoints. Hematocrit and erythrocyte counts were significantly depressed in both sexes at the high dose only (-7.2% and 7.6% in males and females respectively for hematocrit and -5.4% and 4.4% for males and females respectively for red blood cell counts). Hemoglobin was also significantly decreased in high dose males only (-6.4% compared to controls). Serum blood urea nitrogen was significantly increased in both sexes at the high dose only (+76% in males, +79.6% in females) as was creatinine (+38% in both sexes) sodium (+1.4% in males, +0.7% in females) and chloride (+2% in males, + 2.9% in females). Urine volume was significantly increased in both sexes at the high dose (+227% in males and +147% in females compared to controls). Urine volume was also significantly increased in males in the 300 ppm group (+38% compared to controls). Kidney weights - absolutely and relative to both body and brain weight - were significantly increased in both sexes at the high dose only. Kidney weights relative to body weight were increased 44% in males and 45% in females compared to controls.

Kidney alterations at both gross necropsy and at histopatholgy were seen in both the 300 and 600 ppm dose groups. Toxic nephrosis classified as "minimal" was seen in 7 of 15 males and 11 of 15 females at 300 ppm. Toxic nephrosis classified as severe was see all animals of both sexes at the high dose. Tubule crystals were seen in 10 of 15 males and 11 of 15 females at the high dose.
There were no compound- related effects in mortality, clinical signs, or ophthamology in either sex at any dose.

The LOAEL is 21 mg/kg/day based on kidney alterations . The NOAEL is 6.8 mg/kg/day.

This subchronic toxicity study is classified **Acceptable-Guideline** and does satisfy the guideline requirement for a subchronic oral study (870.3100) in the rat.

870.3700 - Developmental toxicity in rats

In a developmental toxicity study (MRID 41065202) hydroxyatrazine, 97.6% a.i., was administered to 104 Sprague-Dawley females 26/dose by gavage at dose levels of 0, 5, 25, or 125 mg/kg/day from days 6 through 15 of gestation.

There were no maternal deaths in this study. Two of the 22 pregnant females in the high dose group had enlarged, mottled kidneys, findings which were not seen in any other animal and are considered compound-related. Food consumption was significantly decreased (8.7%; $p<0.05$), at the high dose group only, during the 6-16 day dosing period. Body weight and body weight gain were unaltered by atrazine treatment. There were no clinical signs attributable to compound exposure. There were no alterations in cesarean section parameters in dose groups *vs* controls.

The maternal LOAEL is 125 mg/kg/day, based on decreased food consumption during the dosing period and enlarged and mottled kidneys. The maternal NOAEL is 25 mg/kg/day.

Fetal death and resorptions were not altered by compound exposure. The high dose group only displayed increases in partially ossified interparietals (control fetal/litter incidence of 20%/56% *vs* high dose fetal/litter of 44%/91%); partially ossified hyoid (control fetal/litter 6%/20%, *vs* 17%/55%). Mean male and female fetal body weight at the high dose was significantly ($p<0.05$) reduced compared to controls (Control male/female body weight 3.6/3.4 grams *vs* male/female high dose 3.5/3.3 grams. There were no increases in any visceral findings in dosed fetuses.

The developmental LOAEL is 125 mg/kg/day, based on increased incidence of partially ossified interparietal and hyoid bones and decreased fetal body weight. The developmental NOAEL is 25 mg/kg/day.

The developmental toxicity study in the rat is classified **Acceptable-Guideline** and <u>does</u> satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3a) in the rat.

870.4100 (870.4300) Chronic Toxicity – Rat

In a 2-year combined chronic feeding/carcinogenicity study (MRID 43532001), G-34048 technical (hydroxyatrazine) of 97.1% purity was administered in the diet to groups of 70 or 80 male and 70 or 80 female Crl:CD (SD) BR strain rats at dose levels of 0 (control), 10, 25, 200 or 400 ppm (equivalent to 0, 0.388, 0.962, 7.75, or 17.4 mg/kg/day in males and to 0, 0.475, 1,17, 9.35, or 22.3 mg/kg/day in females). Ten or 20 rats/sex/group were sacrificed at 12 months; the remaining 60 animals in each group were scheduled to be sacrificed at 24 months. Due to high mortality in the 400 ppm group, however, the surviving males and females in the 400 ppm group were sacrificed at 18 months. Mortality, clinical signs of toxicity, body weights, food consumption and water consumption were monitored. Ophthalmologic examinations were performed. Hematological examinations, clinical chemistries and urinalysis were also performed. Necropsy examinations were conducted on all animals and organ weight determinations were made on all animals sacrificed at 12, 18, 24 months. Histopathological examinations were made on a complete set of organs/tissues from all animals in the control, 200 and 400 ppm groups. Histopathological examinations were also performed on a limited set of organs/tissues from the 10 and 25 ppm groups.

At 400 ppm, an excessive treatment-related mortality was observed for both males and females and this dose level was terminated at 18 months. Severe renal failure was the predominant cause of death for these animals. Prior to death or sacrifice, these animals exhibited emaciation, dehydrated bodies, pallor and other clinical signs of toxicity expected in animals with severe renal failure. Greatly decreased body weights, body weight gains and food consumption were observed in these animals throughout the study (to 18 months). Water consumption was increased during the first year of the study. Changes in hematology parameters (including anemia), in clinical chemistries parameters (indicating renal disturbances) and in urinalysis parameters (including crystalline material in urine samples) were observed in 400 ppm males and females. Gross necropsies, organ weights and histopatholgy indicated that kidney and lower urinary tract were the primary target organs in both males ands females at 400 ppm. Kidney effects included discoloration, calculi and rough pitted surfaces seen at gross necropsy; increased kidney weights; and severe histopathological changes including deposition of crystalline material within collecting ducts and renal pelvises, calculi, other morphological changes and accelerated chronic progressive nephropathy. In addition, secondary effects in extra renal tissues reflected the severe renal damage and resulting renal failure in these animals.

At 200 ppm, similar but less severe gross and histopathological effects on the kidneys were observed in both males and females. Secondary effects in extra renal tissues were generally not observed at this dose level. At 25 ppm, no treatment-related effects were observed in either male or female rats. An accumulation of interstitial matrix in the papilla of the kidneys of female rats was observed at this dose level, but the toxicologic significance of this observation, in the absence of any other signs of renal damage or impaired renal function, was highly questionable.

A treatment-related increased incidence of tumors of any type was not observed in the treated male or female animals in this study. In particular, there was no increase above control levels in the incidence of mammary gland tumors in either males or females. In addition, onset times for mammary gland tumors in female rats were not decreased in this study.

The LOAEL is 8.7 mg/kg/day based on gross and histopathological effects in the kidneys. The NOAEL 1.0 mg/kg/day.

This study is classified as **Acceptable-Guideline** and <u>does</u> satisfy the 870.4300 guideline requirement for a combined chronic/carcinogenicity toxicity study.

870.5100 Bacterial Reverse Mutation Assay

The atrazine metabolite hydroxyatrazine (99% purity) failed to induce reverse mutation (as evidenced by no increase over controls in revertant colony counts) in Ames testing at concentrations up to the limit dose of 5000 : g/0.1 mL. This study is classified **Acceptable-Guideline** (MRID 40722304).

870.5385 Mammalian Bone Marrow Chromosome Aberration Test

The atrazine metabolite hydroxyatrazine (99% purity) did not induce an increase in micronuclei in mice fed concentrations up to the limit dose of 5000 mg/mL. This study is classified **Acceptable-Guideline** (MRID 41479401).

870.5550 Other genetic effects

The atrazine metabolite hydroxyatrazine (96-99% purity) was negative for inducing unscheduled DNA synthesis in primary rat hepatocytes up to and beyond precipitating concentrations (> 500 : g/mL). This study is classified **Acceptable-guideline** (MRID 40422305).

870.5550 Other genetic effects

The atrazine metabolite hydroxyatrazine (96-99% purity) was negative for inducing unscheduled DNA synthesis in human fibroblasts (CRL 1521) up to and beyond precipitating concentrations (> 500 : g/mL). This study is classified **Acceptable-guideline** (MRID 40888101).

5.0 TOXICITY ENDPOINT SELECTION

5.1 See Section 8.2 for endpoint selection table

5.2 Dermal Absorption

The maximum percentage of atrazine absorbed in the rat study after a 10 hr (representative of a typical workday) exposure was 21.6% (rounded up to 22%). The maximum percent absorbed after any duration of exposure, in the human dermal penetration study described above under section 4.8, was 5.6% (rounded up to 6%). Because the maximum percent absorbed is being used and because an ample amount of time (168 hours) was allowed for absorption to occur, 6% is deemed to be a protective estimate of dermal penetration in the human.

Because both rat and human dermal absorption studies are available, a "Rat: Human Dermal Penetration Factor" can be calculated. The available data indicates that skin permeability of atrazine is lower in humans than in rats. To account for this species difference, dermal NOAELs derived from rat studies may be multiplied by a Rat: Human dermal penetration factor. The Rat: Human dermal penetration factor is calculated by dividing the dermal absorption in the rat by the dermal absorption in the human.

$$\text{Rat: Human Dermal Penetration Factor} = \frac{22}{6} = \mathbf{3.6}$$

This factor is multiplied by the NOAEL for exposure scenarios in which endpoints from a dermal study are used. Exposure scenarios whose endpoints are derived from oral studies will use a 6% dermal absorption factor.

5.3 Classification of Carcinogenic Potential

5.3.1 Conclusions

Numerous studies, both guideline studies and non-guideline studies submitted by the registrant as well as studies performed by EPA's NHEERL [described in this document] examine the carcinogenicity and mode of action of atrazine. In a series of meetings [October 13, 1999 , April 12, 2000, and October 19, 2000] held subsequent to the Fifth HED Cancer Peer Review Committee (CARC) of July, 1996, the CARC met to examine these studies. The data pertaining to atrazine's carcinogenicity and mode of action were also examined by the FIFRA Science Advisory Panel (SAP) on June 27th to June 29th, 2000. Both the CARC and SAP agreed that atrazine is associated with mammary and pituitary tumor formation in the female SD rat, but not in male SD rats or CD-1 mice and F-344 rats of either sex. CARC and SAP also agreed that there are sufficient data available to support a proposed mode of action (MOA) to account for the tumors seen in SD female rats and that this MOA would likely not result in carcinogenicity in humans. Hypothalamic neuronal - GnRH pulse modulation of pituitary releases of LH is a central driver of ovulation in the SD female rat, and atrazine is essentially accelerating the aging process of the CNS control of ovulation, which leads to a constant state of estrus (anovulation) and prolong exposure to estrogen and prolactin. Although there are certain similarities in the control of the hypothalamic-pituitary- ovarian axis between humans and rats in that the hypothalamus can play a key regulatory role in primates, there are fundamental differences. For example, unlike the SD rat, CNS modulation is not the driving factor on human GnRH and LH releases. Human conditions of anovulation are associated with aberrant GnRH and LH pulsatile releases. Even if atrazine induced anovulation in humans like in the SD rat, there is no evidence for the potential of an unopposed estrogen condition that would lead to tumor development. It appears that when LH is low, such as in hypothalamic amenorrhea (HA), a state of low serum estrogen is found, not elevated or prolong estrogen exposure. It should be noted that there is no known cancer risk associated with HA patients, albeit they are at risk to a number of other clinical conditions (e.g., osteoporosis, heart disease, infertility). Another condition of anovulation, polycystic ovarian syndrome (PCOS), is also not a good model for atrazine cancer mode of action in SD rats. The etiology of PCOS is multifactorial, and LH secretion is elevated due to increased synthesis of androgen and their conversion to estrogens. Although atrazine might cause adverse effects on hypothalamic-pituitary function in humans, the hormonal environment conducive to tumor development (i.e., elevated or prolonged exposure to estrogen or prolactin) that is found in SD rats cannot be established in humans. Therefore, it is unlikely that atrazine's mode of cancer action in SD rats is operative in humans.

5.3.2 Classification Carcinogenic Potential

It was the conclusion of the CARC [HED Document 014431; December 13, 2000 meeting] that atrazine should be classified as a '**Not Likely To Be Carcinogenic To Humans**".

Atrazine is associated with mammary and pituitary tumors in female Sprague-Dawley (SD) rats, but not in male SD rats, or either sex of Fischer 344 (F-344) rats or CD-1 mice. Mutagenic and estrogenic activity do not appear to play a significant role in atrazine-associated carcinogenicity. Biological plausibility has been established for the mode of carcinogenic activity of atrazine. The rat cancer mode of action (MOA) involves a process consisting of modulation of the gonadotrophin releasing hormone (GnRH) pulse, attenuation of pituitary releases of luteinizing hormone (LH), and alteration of ovulatory cycles, expressed as constant estrus, which leads to prolonged exposure of mammary and pituitary tissues to estrogen and prolactin, and development of tumors in response to the prolonged hormone exposures. This MOA essentially accelerates the normal aging process in female SD rats. It would be expected to be operative in other rat strains with a similar reproductive aging process (e.g. Long Evans and Wistar). Although atrazine might cause adverse effects on hypothalamic-pituitary function in humans, the hormonal environment conducive to tumor development (i.e., elevated or prolonged exposure to estrogen and prolactin) that is found in SD rats is not expected to occur in humans. Instead, humans respond to reduced LH by having reductions in estrogen and prolactin. Although possible associations between atrazine exposure and non-Hodgkins lymphoma (NHL) and ovarian cancer have been reported in a few epidemiology studies, there is no supporting evidence or a sound argument of biological plausibility that these cancers may result from exposure to atrazine. Also, the lack of multiple confirming studies indicates that the human investigations by themselves do not make a strong case for an association between atrazine exposure and human cancer.

5.3.3 Quantification of Carcinogenic Potential

Not applicable as atrazine is classified as a "Not Likely To Be Carcinogenic To Humans".

6.0 FQPA CONSIDERATIONS

6.1 Special Sensitivity to Infants and Children

There was no evidence of increased sensitivity/susceptibility in two rat and one rabbit developmental toxicity studies using atrazine or in a rat developmental toxicity study using deisopropyl atrazine or a rat developmental toxicity study using deethyl atrazine. There was no evidence of increased sensitivity/susceptibility in a two-generation reproduction study using atrazine, although the available study was performed using the old guidelines, which do not provide for the measurement of parameters that assess the potential for neuroendocrine alterations. There was evidence of increased sensitivity/susceptibility in a developmental toxicity study using DACT. In this study the maternal LOAEL/NOAEL was 75/25 mg/kg/day based on decreased body weight gain during dosing. The developmental LOAEL/NOAEL in this study was 25/2.5 mg/kg/day based on increases in incidence of incompletely ossified parietals, interparietals and unossified hyoids.

The hormonal alterations described above under section 5.3.1 as being the MOA for atrazine-associated carcinogenesis, would also be expected to be associated with reproductive/ developmental toxicity. Indeed, evidence of reproductive/developmental toxicity is also seen in studies performed by EPA's NHEERL labs. These studies are discussed above under section 4.9.5. Atrazine exposure to nursing dams 1-9 days following parturition, in NHEERL studies, was found to result in an increased incidence of prostate inflammation in male offspring 120 days later. The NOAEL for this effect was 12.5 mg/kg/day. Atrazine exposure to male and female rats prior to puberty was found to delay the onset of puberty in both sexes. The NOAEL for this effect in females was found to be 25 mg/kg/day and the NOAEL for males was found to be 6.25 mg/kg/day.

HED is requesting that additional research be performed to further examine and characterize reproductive/developmental effects associated with the above-described MOA that may be resulting from atrazine exposure.

HED is requiring that a new two-generation reproduction study using DACT be performed so as to provide confirmatory information further investigating the apparent increased sensitivity/susceptibility seen in the DACT rat developmental toxicity study. This new study should be performed under the OPPTS Series 870.3800 guideline and measurement of additional parameters not typically examined in this type of study, but relevant to atrazine's demonstrated neuroendocrine alterations, should be considered.

Because of the concerns arising from the NHEERL studies and the increased sensitivity/ susceptibility seen in the DACT study, the FQPA 10X safety factor was retained.

6.2 Recommendation for a Developmental Neurotoxicity Study

A developmental toxicity study is not available and HIARC did not recommend that one be performed.

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8.0 APPENDICES

8.1 Toxicity Profile Summary Tables

8.1.1 Acute Toxicity Table for Atrazine

Acute Toxicity Data of Technical Atrazine

Guideline No.	Test	Results	Tox. Cat.
870.1000	Oral LD_{50} - rat	LD_{50} > 1,869 mg/kg (M&F combined)	III
870.1200	Dermal LD_{50} - rat	LD_{50} > 2,000 mg/kg (M&F combined)	III
870.1300	Inhalation LC_{50} - rat	LC_{50} > 5.8 mg/L (M&F combined)	IV
870.1400	Eye Irritation - rabbit	PIS = 0.0/110	IV
870.1500	Dermal Irritation - rabbit	PIS = 0.2/8.0	IV
870.1600	Dermal Sensitization - guinea pigs	Non-sensitizing	---
870.6100	Acute Delayed Neurotoxicity	Not Required	---

PIS=Primary Irritation Score

8.1.2 Subchronic, Chronic and Other Toxicity Table for Atrazine

Subchronic, Chronic and Other Toxicity Table. Only studies considered acceptable by HED are shown.

Guideline No./ Study Type	MRID No. (year) /Doses	Results
870.3100 90-Day oral toxicity rodents	44723701 (1994) 0, 10, 50, or 500 ppm 0, 0.6, 3.3, 34.0 mg/kg/day - males 0, 0.659, 3.35, 35.3 mg/kg/day - females.	NOAEL = 3.30 mg/kg/day LOAEL = 34.5 mg/kg/day based on decreased body weight
870.3150 90-Day oral toxicity in nonrodents (dogs)	---	Study waived because an acceptable chronic oral study in the dog is avaible.

Guideline No./ Study Type	MRID No. (year) /Doses	Results
870.3200 21/28-Day dermal toxicity	42089902 (1989) 0, 10, 100 or 1000 mg/kg/day	NOAEL = 100 mg/kg/day LOAEL = 1000 mg/kg/day based on statistically significant reductions in food consumption, mean body weight, and percent weight gain in both sexes, statistically significantly increased absolute and relative spleen weights in both sexes
870.3250 90-Day dermal toxicity	---	Study not required
870.3465 90-Day inhalation toxicity	---	Study not required
870.3700a Prenatal developmental in rodents	40566302 (1984) 0, 10, 70, or 700 mg/kg/day	Maternal NOAEL = 10 mg/kg/day. Maternal LOAEL = 70 mg/kg/day, based on reduced body weight gain Developmental NOAEL = 10 mg/kg/day Developmental LOAEL = 70 mg/kg/day based on delayed or no ossification at several sites
870.3700a Prenatal developmental in rodents	41065201 (1989) 0, 5, 25, 100 mg/kg/day	Maternal NOAEL = 25 mg/kg/day. Maternal LOAEL = 100 mg/kg/day based on reduced body weight gain and food consumption. Developmental NOAEL = 25 mg/kg/day. Developmental LOAEL = 100 mg/kg/day based on increased incidence of delayed ossification of skull bones.
870.3700b Prenatal developmental in nonrodents	00143006, 40566301 (1984) 0, 1, 5, or 75 mg/kg/day	Maternal NOAEL = 5 mg/kg/day Maternal LOAEL = 75 mg/kg/day based on decreased body weight, food consumption and increased incidence of clinical signs Developmental NOAEL = 5 mg/kg/day Developmental LOAEL = 75 mg/kg/day based on reduced litter size, increased resorptions and delayed ossification.

Guideline No./ Study Type	MRID No. (year) /Doses	Results
870.3800 Reproduction and fertility effects	40431303 (1987) 0, 10, 50,and 500 ppm 0, 0.75, 3.78, 39.0 mg/kg/day - males 0, 0.86, 3.70, 42.8 mg/kg/day - females.	NOAEL = 3.78 mg/kg/day LOAEL = 39 mg/kg/day in males based on decreased body weights, body weight gains and food consumption Offspring NOAEL = 3.78 mg/kg/day Offspring LOAEL = 39 mg/kg/day based on decreased body weights in both generations of males at PND 21.
870.4100a Chronic toxicity rodents	40629302 (1986) 0, 10, 70, 500, 1000 ppm, 0, 0.5, 3.5 25, 50 mg/kg/day	This guideline satistifed by 870.4300 Combined chronic toxicity/carcinogencity NOAEL = 3.5 mg/kg/day LOAEL = 25 mg/kg/day, based on reduced body-weight gain and food consumption See below under 870.4300 for details
870.4100b Chronic toxicity nonrodents (dogs)	40431301 (1987) 0, 15, 150, 1000 ppm 0, 0.5, 5.0 33.7 mg/kg/day	NOAEL = 5.0 mg/kg/day LOAEL = 33.7 mg/kg/day based on cardiac effects.
870.4200 Carcinogenicity (rat, Fischer-344)	42227001 (1992) 0, 10, 70, 200, 400 ppm 0, 0.5, 3.4, 9.9, 20.2 mg/kg/day - males 0, 0.6, 4.4, 12.7, 26.2 mg/kg/day - females	NOAEL = 3.4 mg/kg/day -males; 4.4 mg/kg/day - females LOAEL = 9.9 mg/kg/day - males; 12.7 mg/kg/day - females based on decreased body weight gain There was no treatment-related increase in tumor incidence when compared to controls. This study used Fischer- 344 rats. The purpose was to demonstrate a lack of tumor repsonse in this strain following atrazine exposure.
870.4200 Carcinogenicity (mice)	40431302 (1987) 0,10,300,1500, 3000 ppm 0, 1.4, 38.4, 194, 385.7 mg/kg/day - males 0, 1.6, 47.6, 246.9, 482.7 mg/kg/day - females	NOAEL = 43 mg/kg/day LOAEL = 222.0 mg/kg/day based on decreased body weight gain in both sexes and increased cardiac thrombi in the females. No evidence of carcinogenicity was seen.

Guideline No./ Study Type	MRID No. (year) /Doses	Results
870.4300 Combined chronic toxicity/ carcinogencity (rat, Sprague-Dawley)	40629302 (1986) 0, 10, 70, 500, 1000 ppm, 0, 0.5, 3.5 25, 50 mg/kg/day	NOAEL = 3.5 mg/kg/day LOAEL = 25 mg/kg/day ,based on reduced body weight gain and food consumption. Mammary tumors increased at 3.5 mg/kg/day and above. This study used the Sprague Dawley strain of rat.
870.5100 Bacterial reverse mutation assay	00060642 (1977) 0, 50, 100, 500, 1000, 5000 µg/plate	Negative in strains TA 98, 100, 1535 and 1537 of *S. typhimurium* up to the limit concentration of 5000 µg/plate both with and without activation
870.5100 Bacterial reverse mutation assay	40246601 (1986) 0, 20, 78, 313, 1250, and 5000 µg/plate	Negative in strains TA 98, 100, 1535, 1537 and 1538 of *S. typhimurium* up to the limit concentration of 5000 µg/plate both with and without activation
870.5385 Micronucleus assay	40722301 (1988) 562.5, 1175, 2250 mg/kg	Negative in doses which induced death in Tif:MAGF mice.
870.5450 Rodent dominant lethal assay	42637003 (1993) 0, 500, 1000, 2000, or 2400 mg/kg	Negative in Tif: MAGf mice at doses 2400 mg/kg.
870.5550 UDS assay	00161790/40722301 (1984) 0, 1.2, 6, 30, 150 : g/ml	Negative in primary rat hepatocytes up to 150 : g/ml
870.5550 UDS assay	42547105 (1992) 0, 15, 46, 139, 417, 835, 1670 µg/ml	Negative in primary rat hepatocyte cultures up to and beyond a dose which caused precipitiation (139 µg/ml)
870.6300 Developmental neurotoxicity		

Guideline No./ Study Type	MRID No. (year) /Doses	Results
870.7485 Metabolism and pharmacokinetics	40431304 (1987) 0, 1, and 100 mg/kg for a single dose given through oral gavage. 1.0 mg/kg/day for 15 days by oral gavage.	*Distribution, accumulation* Distribution was dose-dependent and independent of sex. Distribution appeared to follow first-order kinetics and the half-life in the tissues was 31.3 hours. *Excretion* Approximately 95% of the atrazine excreted within 7 days of dosing. Urinary route accouted for about 75% of the excretion feces accounted for 20%. Route of excretion did not seem to vary among sexes or with dose.
870.7485 Metabolism and pharmacokinetics	MRID 40431305 (1987) The animals were dosed daily for 10 days through a stomach tube with dose levels of 0, 1, 3, 7, 10, 50 or 100 mg/kg/day.	*Distribution, accumulation* Distribution was highest in the red blood cell, followed by the liver, ovary and kidney. When the dose increased the amount distributed in the tissues increased. The distribution appeared to follow first-order kinetics and the tissue half-life was 38.6 hours. This indicates that atrazine, with possible exception of the red blood cell, does not bioaccumulate.
870.7485 Metabolism and pharmacokinetics	MRID 40431306 (1987) Rats were given test 100 mg/kg article was given through the stomach tube in a single oral dose. Other rats were given 16.18 and 19.64 mg/kg and urine was collected over a 24 hr period. The urine was analyzed for metabolites.	*Excretion* In the rats given 100 mg/kg greater than 100% of the administered radioactivity was recovered within 3 days of dosing. Urine was found to contain 47.3% of the radioactivity and the feces 49.3%. The tissues contained 5.75% and 1.4% was found in the blood. *Metabolism* Metabolites indicate that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alky substituents of atrazine appears to be of minor metabolic importance.

Guideline No./ Study Type	MRID No. (year) /Doses	Results
870.7485 Metabolism and pharmacokinetics	MRID 42165503 (1993) Fecal and urinary samples from rats exposed in a seprate metabolism study (MRID 40431304) were obtained and analyzed to determine metabolism profiles.	*Metabolic profile* No sex differences in metabolic profiles were evident. The major fecal metabolite was DACT which accounted for 40% of the total fecal radioactivity.
870.7485 Metabolism and pharmacokinetics	MRID 44713802 (1993) single oral dose of 1 or 100 mg/kg through oral gavage	*Distribution, accumulation* Time to maximum blood concentration (t_{cmax}) was 2 hours and 24 hours for the low and high dose groups, respectively. With exception of red blood cells, whole blood, and skeletal muscle, tissue burden for any specific tissue or organ represented less than 1% of the administered dose by 14 days post dosing *Excretion* Urinary excertion was 64.72% of the total administered low dose over a 48-hour period and 66.16% of the total administered high dose over a 168-hour period. Within 48 hours urinary excretion was 100% and 94% complete for the low-dose and high-dose group, respectively. Fecal elimination accounted for 10.80% and 19.69% of the total dose for the low and high dose groups, respectively.
870.7600 Dermal penetration - *rat*	43314302 (1994) 0.01, 0.1 or 1 mg/cm^2	The percent absorbed increased with exposure time and decreased with dose. Regardless of the dose or exposure time, the majority (65 - 95%) of the radio labeled atrazine was recovered in the washes or was found associated with the skin at the site of exposure.The maximum percentage of atrazine absorbed in the rat study after a 10 hr (representative of a typical workday) exposure was 21.6%.

Guideline No./ Study Type	MRID No. (year) /Doses	Results
870.7600 Dermal penetration - *human*	44152114 (1996) 0.17 and 2.0 mg of [14C] atrazine	The majority (91.1-95.5%) of the dose was not absorbed. After 168 hours, only 5.6% of the dose was absorbed and excreted in the urine and feces of the low-dose group and only 1.2% in the high-dose group.
Special study - Assays of direct estrogenic activity	43598617 (1994) This MRID number describes more than one assay. The doses varied depending on the assay. See above under section 4.9.1 for specific details on doses used.	This study performed a trio of assays: uterotrophic response assay; progesterone receptor competive binding assay; and, a uterine thymidine incorporation assay. All doses tested displayed a lack of clear effects on uterine weight, progesterone binding capacity, and thymidine incorporation. This indicates that atrazine (and DACT and simazine, which were also tested) do not exhibit direct estrogenic activity.
Special study - Assays of direct estrogenic activity	43598618 (1994) This MRID number describes one type of assay conducted under a variety of experiemental conditions. The doses varied. See above under section 4.9.1 for specific details on doses used.	This study describes a series of estrogen receptor competitive binding assays, both *in vivo* and *in vitro*. Overall the results indicate that atrazine (and DACT and simazine, which were also tested) do exhibit some competitive binding with estradiol but only under conditions which favor binding.
Special study - Assays of direct estrogenic activity	43598619 (1995) This MRID number describes more than one assay. The doses varied depending on the assay. See above under section 4.9.1 for specific details on doses used.	This study describes four seperate assays: competive binding assay with the hepatocyte Ah receptor; MCF-7 cell proliferation; gel electrophoresis mobility shift assay using the progesterone receptor; and, luciferase reporter gene assay in MCF-7 cells. Neither atrazine (nor simazine, which was also tested) displayed estrogenic activity or interacted with the Ah receptor in the set of experiments described in this paper.

Guideline No./ Study Type	MRID No. (year) /Doses	Results
Special study - Assays of direct estrogenic activity	43934403 (1995) This MRID number describes more than one assay. The doses varied depending on the assay. See above under section 4.9.1 for specific details on doses used.	This study described several assays, both *in vivo* and *in vitro*. *In vivo* assays: uterine weight, progesterone receptor levels and uterine peroxidase. *In vitro* assays: MCF-7 cell proliferation; gel electrophoresis mobility shift assay using the progesterone receptor; luciferase reporter gene assay in MCF-7 cells; and, a selective medium growth assay in yeast cells. The results of these experiments indicate that *in vivo* atrazine and simazine may exhibit some antiestrogenic activity but no estrogenic activity either *in vivo* or *in vitro*.
Special study - Estrous Cycle and LH Surge Measurements (Pilot study)	43934404 (1996) atrazine not given	This study was a pilot study in which the validity of a proposed protocol for testing the effect of atrazine exposure on the proestrus afternoon luteinizing hormone (LH) was evaluated. This study demonstrated that the proposed protocol adequatyl tested the parameters to examined.
Special study - Estrous Cycle and LH Surge Measurements (28 day exposure)	43934406 (1996) 0, 2.5, 5, 40 and 200 mg/kg/day	NOAEL = 5 mg/kg/day. LOAEL = 40 mg/kg/day based on decreases in food consumption, body weight, body weight gain, estrous cycle alterations and LH surge attenuation
Special study - Estrous Cycle and LH Surge Measurements (6 month exposure)	44152102 (1996) 0, 25, 50, and 400 ppm 0, 1.8, 3.7, 29.4 mg/kg/day	NOAEL = 1.8 mg/kg/day LOAEL = 3.7 mg/kg/day based on estrous cycle alterations and LH surge attenuation
Special study[1]- Hormone and estrous cycle measurements in SD rats	42085001, 42743902 (1991, 1993) *See also 43598622* 0, 70, 400 ppm 0, 4.2, 26.2 mg/kg/day Ten females per dose were sacrificed at 1, 3, 9, 12, 15, 18, 24 mo.	NOAEL = none found LOAEL = 4.2 mg/kg/day based on estrous cycle alterations

Guideline No./ Study Type	MRID No. (year) /Doses	Results
Special Study [1]- Mammary Gland and Ovarian Histomorphology in SD rats	43598622 (1995) *See also 42085001, 42743902* 0, 70, 400 ppm 0, 4.2, 26.2 mg/kg/day Ten females per dose were sacrificed at 1, 3, 9, 12, 15, 18, 24 mo.	NOAEL = none found LOAEL = 4.2 mg/kg/day based early onset of anovulation as indicated by ovarian histomorphology and early onset of indicators of prolonged/increased hormone exposure in the mammary gland
Special study[2] - Two-year bioassay in <u>F-344 rats</u>	42146101 (1991) *See also 4274392 and 43598622* 0, 0.7, 4.8, 14, 33.4 mg/kg/day Ten females per dose were sacrificed at 1, 3, 9, 12, 15, 18, 24 mo.	NOAEL = 14 mg/kg/day LOAEL = 33.4 mg/kg/day based on decreased body weight gain. There was not an increased incidence of any tumor type, nor an early onset of mammary tumors.
Special study[2] - Hormone and estrous cycle measurements in <u>F-344 rats</u>	42743902 (1993) *See also 42146101 and 43598622* 0, 0.7, 4.8, 14, 33.4 mg/kg/day Ten females per dose were sacrificed at 1, 3, 9, 12, 15, 18, 24 mo.	NOAEL = at least 33.4 mg/kg/day LOAEL = none found This study examined hormones and estrous cycles in the animals used in 42146101. The estrous cycle evaluations from this study are deemed unreliable by HED. The ovarian histomorphology data from MRID 43598622 is used to confirm a lack of effect of atrazine treatment on estrous cycles. The animals exhibited hormone levels indiciative of normally aging females of the strain. Exposure to atrazine had no effect on hormone levels.
Special study[2]- Mammary Gland and Ovarian Histomorphology in <u>F-344 rats</u>	43598622 (1995) *See also 42146101 and 42743902* 0, 0.7, 4.8, 14, 33.4 mg/kg/day Ten females per dose were sacrificed at 1, 3, 9, 12, 15, 18, 24 mo.	NOAEL = at least 33.4 mg/kg/day LOAEL = none found This study examined histomorphology in the animals used in 42146101. The animals exhibited ovarian and mammary gland histomorphology indiciative of normally aging females of the strain. Exposure to atrazine had no effect on these histomorpologic parameters.

Guideline No./ Study Type	MRID No. (year) /Doses	Results
Special study - Two-year bioassay with the SD strain of rat	42204401 (1992) 0, 70, 400 ppm 0, 3.8, 23.0 mg/kg/day	NOAEL = 3.8 mg/kg/day LOAEL = 23 mg/kg/day based on decreased body weight gains as well as statistically significant decreases in body weights in the 0-76 week period A statistically significant increase in mammary tumors was seen at the high dose. An early onset of mammary carcinomas was seen.
Special study - Tumor incidence in ovariectomized (OVX) *vs* intact animals	44544701 (1998) 0, 25, 50, 70, 400 ppm 0, 1.5, 3.1, 4.2, 24.4 mg/kg/day - intact 0, 1.2, 2.5, 3.5, 20.9 mg/kg/day - OVX	Intact animal Mammary tumors No mammary tumors seen in any OVX animal
Special study - Long-term estrous cycle measurements	No MRID assigned (1999) 0, 25, 50, 70, 400 ppm 0, 1.5, 3.1, 4.2, 24.4 mg/kg/day	This is the unaudited draft report of the interim estrous cycle data from MRID 44544701 These data demonstrate an early onset of increased % days in estrus in atrazine-treated animals compared to controls.
Special study- Direct comparison of LH surge attenuation of atrazine, simazine, DACT	No MRID assigned (2000) 0, 2.5, 5, 40, 200 mg/kg/day	This is the unaudited draft report of a study in which SD females were exposed to atrazine, simazine or DACT for 28 days and the abilty of these chemicals to attenuate the LH surge was measured. These data showed that simazine and DACT are able to attenuate the LH surge at doses similar to those at which atrazine attenuates the surge.

[1] This study in SD rats contains three parts submitted under three seperate MRID numbers:

MRID 42085001 contains the results of the animal bioassay part of this study (the clinical observations, body weights, food consumption, gross pathology, *etc*.);
MRID 42743902 contains the results of the vaginal smears/estrous cycle determinations and serum hormone measurements;
MRID 43598622 contains the results of the histomorphologic analysis.

[2] This study in F-344 rats contains three parts submitted under three seperate MRID numbers:

MRID 42146101 contains the results of the animal bioassay part of this study (the clinical observations, body weights, food consumption, gross pathology, *etc*.);
MRID 42743902 contains the results of the vaginal smears/estrous cycle determinations and serum hormone measurements;
MRID 43598622 contains the results of the histomorphologic analysis.

8.1.3 Toxicity Table for *DACT*. All avaiable acceptable toxicity studies are shown.

Guideline No./ Study Type	MRID No. (year) /Doses	Results
870.3100 90-Day oral toxicity rodents	43013207 (1991) 0, 0, 10, 100, 250, or 500 ppm 0, 0.7, 6.7, 16.7, or 34.1 mg/kg/day - males 0, 0.7, 7.6, 19.7, or 40.2 mg/kg/day -females.	Male NOAEL= 16.7 mg/kg/day LOAEL= 34.1 mg/kg/day based on decreased body weight and body weight gain Female NOAEL= 0.7 mg/kg/day LOAEL=7.6 mg/kg/day based on estrous cycle alterations
870.3100 90-Day oral toxicity in nonrodents (dog)	41392401 (1990) 0, 5, 100, and 1500/750 ppm (high dose reduced after 6 wks) 0, 0.187, 3.61, and 24.1mg/kg/day -males 0, 0.195, 3.43, and 32.7 mg/kg/day - females	NOAEL = 3.4 mg/kg/day LOAEL = 24.1 mg/kg/day based on cardiac effects and tremors in the males.
870.4100 Chronic toxicity in nonrodent (dog)	41392401 (1990) 0, 5, 100, and 1500/750 ppm (high dose reduced after 6 wks) 0, 0.187, 3.61, and 24.1mg/kg/day -males 0, 0.195, 3.43, and 32.7 mg/kg/day - females	NOAEL = 3.4 mg/kg/day LOAEL = 24.1 mg/kg/day based on cardiac effects and tremors in the males.
870.3700a Prenatal developmental toxicity in rodents	41392402 (1989) 0, 2.5 , 25, 75 or 150 mg/kg/day	Maternal NOAEL = 25 mg/kg/day Maternal LOAEL = 75 mg/kg/day, based on decreased body weight gain during dosing. Developmental NOAEL is 2.5 mg/kg/day. Developmental LOAEL = 25 mg/kg/day, based on increases in incidences of incompletely ossified parietals, interparietals and unossified hyoids.
870.5100 Bacterial reverse mutation assay	40722302 (1987) 0, 20, 78, 313, 1250, 5000 :g/plate	Negative in the *Salmonella*/Ames assay up to limit concentration of 5000 :g per plate in strains TA 98, TA100, TA1535, and TA1537 with and without metabolic activation
870.5550 UDS assay	40722303 (1987) 0, 5.56, 16.67, 50, 150, 300, 600 :g/ml	Negative up to 600 :g/ml (which exceeded the solubility limit of 400 :g/ml) in UDS assay using isolated human fibroblasts without activation only (not performed with activation)

8.1.4 Toxicity Table for *G-28279*. All avaiable acceptable toxicity studies are shown.

Guideline No./ Study Type	MRID No. (year) /Doses	Results
870.100 Acute oral toxicity	43013201 (1991) 500, 2000, 3500, 5050 mg/kg	LD_{50} for both sexes combined= 1240 mg/kg
870.3100 90-Day oral toxicity rodents	MRID 43013205 (1992) 0, 10, 50, or 500 ppm 0, 0.602, 3.20, 34.9 mg/kg/day - males 0, 0.641, 3.34, 37.5 mg/kg - females	NOAEL=3.2 mg/kg/day LOAEL=34.9 based on based on decreased body weights and body weight gains in both sexes. and possible histopathologic effects in the liver, adrenal cortex, and thyroid.
870.3100 90-Day oral toxicity in nonrodents (dog)	MRID 43013203 (1992) 0,15,100, 500, or 1000 ppm 0, 0.6, 3.8, 18.9, 33.4 mg/kg/day -male 0, 0.6, 3.8, 18.0, 33.3 mg/kg/day - female	NOAEL=3.8 mg/kg/day LOAEL=18.9 based on decreased food consumption and body weight gain and decreased organ-to brain weight ratios for the heart, testes, and prostate gland
870.3700a Prenatal developmental in rodents	43013208 (1992) 0, 5, 25, or 100 mg/kg/day.	Maternal NOAEL= 5 mg/kg/day Matenal LOAEL= 25 mg/kg/day based on decreased body weight gain and food consumption Developmental NOAEL= 5 mg/kg/day Developmental LOAEL 25 mg/kg/day based on . increased fetal and litter incidences of fused sternebrae 1 and 2
870.5100 Bacterial reverse mutation assay	43093101 (1990) 0, 313, 625, 1250, 2500, 5000 :g/ml	Negative in the *Salmonella*/Ames and *E. Coli* WP2uvrA assays up to limit concentration of 5000 :g per plate. Tested in *S. Typhimurium* in strains TA 98, TA100, TA1535, and TA1537 with and without metabolic activation
870.5375 Micronucleous assay	43093103 (1991) 0, 120, 240, 480 mg/kg	Negative in Tif:MAGF mice treated up to the maximum tolerated dose of 480 mg/kg
870.5550 UDS assay	43093105 (1991) 0, 7.4, 22.2, 66.6, 200, 400, 800 :g/ml	Negative up to cytoxic concentrations of 800 :g/ml in primary rat hepatocyte cultures.

8.1.5 Toxicity Table for *G-30033*. All avaiable acceptable toxicity studies are shown.

Guideline No./ Study Type	MRID No. (year) /Doses	Results
870.100 Acute oral toxicity	43013202 (1991) 500, 2000, 3500, 5050 mg/kg	LD_{50} for both sexes combined= 1111 mg/kg
870.3100 90-Day oral toxicity rodents	43013206 (1991) 0, 10, 50, or 500 ppm 0, 0.68, 3.2, and 35.1 mg/kg -male 0, 0.72, 3.3, and 38.1 mg/kg /day - female	NOAEL = 3.2 mg/kg/day LOAEL = 35.1 mg/kg/day based on decreased body weight of high dose female rats and decreased food efficiency of high-dose male and female rats.
870.3100 90-Day oral toxicity in nonrodents (dog)	43013203 (1992) 0, 15, 100, or 1000 ppm 0, 0.56, 3.71, 28.85 mg/kg/day - males 0, 0.51, 3.88,32.18 mg/kg/day - females	NOAEL = 3.7 mg/kg/day LOAEL= 28.9 mg/kg/day based on renal tubular hyperplasia/basophilia in 3/4 male and 2/4 female dogs
870.3700a Prenatal developmental in rodents	43013209 (1992) 0, 5, 25, or 100 mg/kg/day	Maternal NOAEL = 5 mg/kg/day Maternal LOAEL = 25 mg/kg/day based decreased food consumption, body weight/weight gain and food consumption Developmental NOAEL = 25 mg/kg/day Developmental LOAEL = 100 mg/kg/day based on increased fetal and litter incidences of fused sternebrae 1 and 2 and increased fetal incidence of poor ossification of the proximal phalanx of posterior digit 5 (a skeletal variation)
870.5100 Bacterial reverse mutation assay	43093102 (1991) 0, 313, 625, 1250, 2500, 5000 :g/ml	Negative in the *Salmonella*/Ames and *E. Coli* WP2uvrA assays up to limit concentration of 5000 :g per plate. Tested in *S. Typhimurium* in strains TA 98, TA100, TA1535, and TA1537 with and without metabolic activation
870.5375 Micronucleous assay	43093104 (1991) 0, 120, 240, 480 mg/kg	Negative in Tif:MAGF mice treated up to the maximum tolerated dose of 480 mg/kg
870.5550 UDS assay	43093106 (1991) 0, 9.25, 27.7, 83.3, 250, 500, 1000 :g/ml	Negative up to cytoxic concentrations of 1000 :g/ml in primary rat hepatocyte cultures.

8.1.6 Toxicity Table for *hydroxyatrazine*. All avaiable acceptable toxicity studies are shown.

Guideline No./ Study Type	MRID No. (year) /Doses	Results
870.3100 90-Day oral toxicity rodents	MRID 41293501 (1989) 0, 10, 100, 300, 600 ppm 0, 0.6, 6.3, 18.9, 37.5 mg/kg/day - males 0, 0.8, 7.4, 22.8, 45.6 mg/kg/day - females	NOAEL = 6.3 mg/kg/day in males and 7.4 mg/kg/day in females LOAEL = 18.9 mg/kg/day in males and 22.8 mg/kg/day in females based on kidney alterations .
870.3700a Prenatal developmental in rodents	MRID 41065202 (1989) 0, 5, 25, or 125 mg/kg/day	Maternal NOAEL = 25 mg/kg/day Maternal LOAEL = 125 mg/kg/day based on decreased food consumption during the dosing period and enlarged and mottled kidneys. Developmental NOAEL = 25 mg/kg/day. Developmental LOAEL = 125 mg/kg/day based on increased incidence of partially ossified interparietal and hyoid bones and decreased fetal body weight.
870.4100a (870.4300) Combined Chronic Toxicity/ Oncogenicity – Rat	MRID 43532001 (1995) 0, 10, 25, 200, 400 ppm 0, 0.39, 1.0, 7.8, 17.4 mg/kg/day - males 0, 0.5, 1.2, 9.4, 22.3 mg/kg/day - females	NOAEL = 1.0 mg/kg/day for males and 1.2 mg/kg/day for females LOAEL = 7.8 mg/kg/day for males and 9.5 mg/kg/day for females based on gross and histopathological effects in the kidneys
870.5100 Bacterial reverese mutation assay	MRID 40722304 (1988) 0, 20, 78, 313, 1250, 5000 :g/0.1 ml	No increases in revertant colonies in TA 98, 100, 1535, and 1537 Salmonella strains exposed to precipitating concentrations (313 :g/plate and above) both with and without activation system.
870.5375 Micronucleous assay	MRID 41479401 (1988) 0, 1250, 2500, 5000 mg/ml	No increase in micronuclei in mice treated with acute intubated doses up to the limit dose of 5000 mg/ml.
870.5550 UDS assay	MRID 40722305 (1988) 0, 13.9, 41.7, 125, 375, 750, 1500 :g/ml	No evidence of unscheduled DNA synthesis was found up to the limits of solubility (increasing precipitation from 500 :g/ml) and at concentrations approaching toxicity (1500 :g/ml) in primary hepatocyte cultures treated *in vitro*.
870.5550 UDS assay	MRID 40888101 (1988) 0, 13.9, 41.7, 125, 375, 750, 1500 :g/ml	Negative up to the limits of solubility (increasing precipitation from 500 :g/ml) and severe cytotoxicity (1500 :g/ml) in human fibroblast cells.

8.2 Summary of the Toxicological Dose and Endpoints for Atrazine Use in Human Risk Assessment[+]

Exposure Scenario	Dose Used in Risk Assessment, UF	FQPA SF and Endpoint for Risk Assessment	Study and Toxicological Effects
Acute Dietary **all populations**	NOAEL= 10 mg/kg/day UF = 100 **Acute RfD** = 0.1 mg/kg/day	FQPA SF = 10 **aPAD** = 0.01 mg/kg/day	Developmental toxicity study in the rat NOAEL = 10 mg/kg/day based on delayed ossification of certain cranial bones at the LOAEL of 70 mg/kg/day (Weight of evidence from MRIDs 4106520, 00143006, 40566301, and 45166902 contributed to the selection of this endpoint)
Chronic Dietary **all populations**	NOAEL= 1.8 mg/kg/day UF = 100 **Chronic RfD** = 0.018 mg/kg/day	FQPA SF = 10 **cPAD** = 0.0018 mg/kg/day	Six-month LH surge study in the rat NOAEL = 1.8 mg/kg/day based on attenuation of LH surge and estrous cycle alterations at LOAEL of 3.65 mg/kg/day.
Incidental Oral, Short-Term	NOAEL= 10 mg/kg/day		Developmental toxicity study in the rat NOAEL = 10 mg/kg/day based on Decreased body weight during the first five days of dosing in the dams
Incidental Oral, Intermediate-Term	NOAEL = 1.8 mg/kg/day		Six-month LH surge study in the rat NOAEL = 1.8 mg/kg/day based on attenuation of LH surge and estrous cycle alterations at LOAEL of 3.65 mg/kg/day.
Short -Term Dermal	NOAEL= 360 (NOAEL from study was 100 mg/kg/day. Multiplied by the rat:human dermal penetration factor of 3.6 = 360 mg/kg/day)		NOAEL = 360 mg/kg/day based on reductions in food consumption, mean body weight, and percent weight gain in both sexes, statistically significantly increased absolute and relative spleen weights in both sexes, and slight changes in excretion (*i.e.* few and/or mucoid feces) seen at LOAEL of 1000 mg/kg/day and multiplied by 3.6 dermal penetration factor.
Intermediate and Long-Term Dermal	NOAEL= 1.8 mg/kg/day Dermal absorption rate = 6%		Six-month LH surge study in the rat NOAEL = 1.8 mg/kg/day based on attenuation of LH surge and estrous cycle alterations at LOAEL of 3.65 mg/kg/day.

Exposure Scenario	Dose Used in Risk Assessment, UF	FQPA SF and Endpoint for Risk Assessment	Study and Toxicological Effects
Short-Term Inhalation	NOAEL= 10 mg/kg/day Inhalation absorption rate = 100%		Developmental toxicity study in the rat NOAEL = 10 mg/kg/day based on delayed ossification of certain cranial bones at the LOAEL of 70 mg/kg/day (Weight of evidence from MRIDs 4106520, 00143006, 40566301, and 45166902 contributed to the selection of this endpoint)
Intermediate and Long-Term Inhalation	NOAEL= 1.8 mg/kg/day		Six-month LH surge study in the rat NOAEL = 1.8 mg/kg/day based on attenuation of LH surge and estrous cycle alterations at LOAEL of 3.65 mg/kg/day.
Cancer (oral, dermal, inhalation)	"Not likely"		Risk assessment for cancer RfD = reference dose not required

[1] UF = uncertainty factor,
NOAEL = no observed adverse effect level
PAD = population adjusted dose (a = acute, c = chronic) adjusted for FQPA safety factor
FQPA SF = FQPA safety factor
LOAEL =lowest observed adverse effect level
RfD = reference dose
MOE = margin of exposure

+: For short and intermediate term residential exposure, MOE≥1000 does not exceed level of concern.
For short and intermediate term occupational exposure, MOE≥100 does not exceed level on concern.